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American Journal of Veterinary Research

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Contributions of Veterinary Research to the American People

THE ESTABLISHMENT of the American Journal of Veterinary Research has much the same significance as laying the cornerstone of a new structure devoted to professional and public service. And to editorialize on such an event has much in common with the dedicatory remarks on such an occasion. The foundations of veterinary research are already broad and deep and enduring. We now prepare to extend them, looking toward future needs.

The contributions of veterinary research to the American people may be said to be four-square in the sense that they group themselves naturally into four main groups. These are: 1) benefits that accrue largely to the veterinary profession and to the animals served by veterinarians; 2) contributions having application in human medicine and exerting a far-reaching, beneficial influence on human health; 3) discoveries of benefit to industries, notably the meat and dairy industries and the production of biological products and disinfectants; 4) substantial contributions to statesmanship with special reference to sound state and national policies on livestock sanitary problems. Consideration of these four fields of influence shows why the veterinary profession, though small numerically in comparison with other learned groups, has had so great a bearing on national welfare.

As a timely illustration, the U. S. Department of Agriculture has just announced the practical eradication of bovine tuberculosis from every county and every state in the United States. This achievement marks the culmination of 23 years of diligent, courageous effort. It affects the health of man and beast, the products of industry, and the exchange of commodities among states and nations. The bovine tuberculosis project is notable for the vast scale of its operations and the necessary effective coördination of man power, supplies, finances and records. The application of more than 232 million tuberculin tests and the removal of nearly 4 million reactors are spectacular results. Yet behind the great field organization was a small group of laboratory workers diligently producing the pure and potent tuberculin so essential for the successful conduct of the project, and while so engaged these workers were able to improve and refine the product, making the so-called new tuberculin, grown on a synthetic medium, about 10 per cent more efficient than the former type.

This example illustrates the intrinsic worth of research in the world today. Far from being a recluse, as so often considered, the research worker is actually the pioneer, blazing a trail for new developments in agriculture and industry. And though seeming to lack enduring substance, research materials such as tissues, chemicals, apparatus and manuscripts combine to form a sound and durable foundation on which some 12,000 veterinarians may base their daily work. Each number of the American Journal of Veterinary Research may be considered as providing additional foundation units.

I trust that authors of research papers, in presenting their findings, will visualize the varied interests to be reached. In making this observation I have in mind the constructive interpretations of veteran re-

search workers who associated their efforts with related practical applications. Smith, Kilbourne and Curtice, for instance, in their classical research on tick fever conducted field surveys and traveled extensively. Thus they were able to develop procedures leading to successful tick eradication. Dalrymple and Butler, in fostering livestock production in the South, emphasized the nutritive value of various agricultural by-products, thereby associating economical farm practices with veterinary phases of livestock production. Ransom and Hall pictured dramatically in professional papers, as well as in their popular writings, the far-reaching benefits of parasite control. Dorset and associates, besides developing anti-hog-cholera serum, sought constantly to improve methods of administering the product in the interests of still more effective mastery over hog cholera. Huddleson, Fitch, Schroeder, Cotton, Buck, Barnes and others in their researches on Bang's disease were responding to the appeals of cattlemen who looked to technical institutions for relief from the ravages of this dangerous infection. Oftentimes also a discovery within a limited field has far-reaching benefits when potential broad applications are discovered and pointed out. Recent research on phenothiazine as an anthelmintic is a case in point.

These examples, of course, are merely a few fruits of veterinary research among others legion in number. With this background of past attainment, veterinary research workers have much to inspire and guide them. I am confident that the pages of this new journal will provide a valuable, needed medium for extending promptly the usefulness of new knowledge in veterinary science.

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The Cultural and Biochemic Properties of Erysipelothrix Rhusiopathiae*

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SWINE ERYSIPELAS, long a serious disease in Europe, has become of importance in North America only within the last decade. The increasing number of reports appearing in the literature of this country indicate that infection with *Erysipelothrix rhusiopathiae* is quite widespread. The disease is not confined to swine, but has been observed in sheep and poultry as well. Infection with this microörganism in man has been reported a number of times in the United States.

Because of the increasing importance of swine erysipelas, it was thought expedient to learn more about the cultural and biochemic characteristics of the etiologic agent. A search of the literature revealed that little work has been done in America with regard to the bacteriology of the disease. European literature contains many reports of studies on *E. rhusiopathiae*, but the results are varied and controversial. This is especially true of the fermentation reactions upon which most laboratories depend for differential diagnosis.

REVIEW OF LITERATURE

Pasteur[1] described rather meagerly a slender bacillus isolated from cases of swine erysipelas. Pasteur and Thuillier[2] used this culture to demonstrate, for the first time, the method of artificial immunization through means of live bacteria attenuated by animal passage. They were able to protect swine against spontaneous infections by injecting cultures of a strain that had been passed through rabbits.

The first accurate description of the swine-erysipelas bacillus was given by Loeffler.[3] He cultivated a slender, short, straight or bent rod from swine showing clinical symptoms of the disease. He found that his cultures would produce true swine erysipelas. Mice injected with the organism showed the symptoms and pathology of mouse septicemia. Gelatin-stab cultures showed the typical test-tube brush growth which had been described for the mouse-septicemia bacillus. Loeffler called attention several times to the close similarity between the microörganism of swine erysipelas and that of mouse septicemia described by Koch[4] and by Löffler.[5]

Salmon,[6] in this country, reported that the vaccine of Pasteur and Thuillier was not a pure culture of the swine-erysipelas bacillus. Salmon mentioned the similarity between the mouse-septicemia bacillus and the microörganism of swine erysipelas. Moore[7] isolated the mouse-septicemia bacillus in this country from a pig which had lesions of an acute septicemia. His cultures were composed of short, slender, Gram-positive rods with an occasional long filamentous form. Gelatin-stab cultures showed the typical test-tube brush growth described by previous authors. Smith[8] described rather completely a similar microörganism from an outbreak of an acute disease of swine in Minnesota. Small Gram-positive rods and long filaments were seen in all cultures. In gelatin a radiating growth resembling a small brush was seen. Tiny transparent colonies developed on solid medium. The fermentation reactions of this culture were variable, but usually dextrose and lactose broths became acid. Smith concluded that this microörganism was closely related to or identical with the bacillus of swine erysipelas.

Rosenbach[9] discussed the causes of erysipeloid in man, swine erysipelas and mouse septicemia. On the basis of morphologic and cultural characteristics he concluded that the three etiologic agents were closely related. He referred to them as *E. erysipeloide*, *E. porci*, and *E. murisepticus*. Many investigators—Prettner,[10] Rickman,[11] Rahm,[12] Zibert,[13] and Zosel[14]—have been unable to find means of differentiating the three species of Rosenbach. Poels,[15] Eber,[16] Meyn,[17] Schmidt,[18] Marsh,[19] Beaudette and Hudson,[20] Klauder and Harkins,[21] and Van Roekel, Bullis, and Clarke[22] have studied the relationships of the microörganisms isolated from humans, sheep, chickens, turkeys and swine without being able to show any significant differences between strains.

Klauder, Righter, and Harkins[23] have isolated bacteria with cultural and morphologic characteristics of the swine-erysipelas bacillus from cases of erysipeloid in fish handlers. Kondo and Sugimura[24] have been able to demonstrate the presence of these microörganisms on the body surface of salt-water fish, houseflies, and in putrefying horse flesh. On the basis of cultural and serologic studies they concluded that

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the strains were identical to a known *E. rhusiopathiae* culture.

The literature concerning the differential characters of *E. rhusiopathiae* is brief, and the reports of individual investigators are varied. Schipp[25] reported that swine-erysipelas bacilli ferment dextrose and lactose, but not sucrose. He stated that Endo's agar, neutral red agar, and Drigalski's medium show no change in 48 hours. TenBroeck[26, 27] reported that his cultures showed no reaction on xylose, dulcitol, maltose, mannitol, saccharose, inulin, salicin, dextrin, starch or glycerol. He suggested that the positive reaction on arabinose may be due to impurities. TenBroeck used heat-sterilized media for his fermentation studies. He found negative tests for indol, ammonia and phenol formation. Hydrogen sulfide tests were readily positive and hemolysis could be observed around deep colonies. Creech[28] reported that cultures isolated from cases of swine erysipelas produced no change in milk or potato and were indol negative. Dextrose, levulose and lactose were fermented with acid but no gas. Arabinose, dulcitol, galactose, inulin, maltose, mannite, raffinose, sucrose, salicin and xylose were all negative for acid and gas. Tests for hydrogen sulfide formation were negative.

Zibert,[13] using serumized broth, reported acid formation from dextrose, lactose, maltose and galactose with slight acid formation on sucrose, levulose, arabinose, mannose, dulcitol and mannitol. Meyn[29] reported essentially the same reactions using serumized broth. Baker[30] reported that *E. rhusiopathiae* gives no reaction in any fermentable media. Deem and Williams[31] and Beaudette and Hudson[20] have also studied the fermentation reactions of this group. They report acid formation from dextrose, lactose, levulose and galactose, and variable results on xylose and arabinose. The former authors found a variable reaction on mannose. More recently Van Roekel, Bullis, and Clarke[22] have reported that arabinose is not fermented and that the reaction on xylose and mannose is variable. Giltner,[32] Cornell and Glover,[33] and Wayson[34] report positive reactions on maltose whereas most workers report that no change is produced. Stiles and Davis[35] have made limited studies and report that only dextrose, lactose, levulose and galactose are attacked with the formation of acid.

Morrill[36] isolated the swine-erysipelas microörganism from cases of erysipeloid in veterinary students and from the equine cadaver which they had been dissecting. These strains are reported to produce acid in dextrose, lactose and sucrose. Rosenwald and Dickinson[37] report that four strains isolated from turkeys acidified broth containing dextrose, galactose, lactose and levulose. One strain also showed acid formation in xylose. Lead-acetate medium was not blackened and only one strain reduced nitrates. In a study of 43 strains Watts[38] reported that all strains produced acid in glucose, maltose, lactose, levulose, starch and dextrin, and that some gave acid reactions on arabinose, dulcitol, glycerol, inulin, mannitol, sucrose, xylose and inositol. Barber[39] reported that only dextrose, lactose, galactose and levulose were fermented with the production of acid.

From this short review it is evident that at present no agreement exists concerning the reactions produced by *E. rhusiopathiae* on differential media. This is especially true of the fermentation reactions. In most cases the method of preparing the medium is not given so that discrepancies can not be explained. TenBroeck,[26, 27] while studying the swine-erysipelas bacilli, found that when serumized broth was used, positive reactions were obtained in maltose, dextrin, starch and sucrose, but when serum was not used, acid was not produced from these carbohydrates. He thought that enzymes in the unheated serum might be responsible for the false positive tests. Newsom and Cross[40] observed that heat sterilization would alter carbohydrates enough to be a source of error in fermentation studies.

Stuart[41] studied the hemolytic properties of the swine-erysipelas bacilli and reported that all strains produce a zone of green hemolysis around deep colonies due to the formation of hydrogen peroxide. He also studied the staining qualities of these bacilli and found that they decolorized with ease and that some strains were definitely Gram negative. This is in contrast to the report of Edwards,[42] who recorded that the swine-erysipelas bacillus is one of the most difficult of all Gram-positive bacteria to decolorize.

Variations in morphology and colony formation have been reported by many workers. Gustine,[43] Wyschelesky,[44] Meyn,[29] Redlich,[45] and Schoening, Gochenour and Grey[46] have shown that both short, slender rods and long, filamentous forms may be seen in cultures of *E. rhusiopathiae*. They report that colonies of two types may be seen on solid media. The so-called smooth colony is small, round and regular in outline with a smooth convex surface whereas the so-called rough colony is larger, irregular in outline, flat and with a rough surface. Sabella[47] reported the formation of involution forms when cultured in the presence of saponin. These were clubbed, coccoid, filamentous or spindle-shaped.

Kettlitz[48] found that growth occurred over a range of hydrogen-ion concentration from pH 7.2 to pH 8.4 with pH 7.6 to pH 8.0 as optimal. Alphen[49] reported that a reaction giving an orange color with phenol red was the proper one for maximum growth of the swine ery-

erysipelas bacillus and that it was very sensitive to changes in the reaction of the medium.

EXPERIMENTAL PROCEDURE

Cultures.—This report is based on 60 different strains obtained from various laboratories in this country. Fifty-four strains were isolated from swine. Some of these had been propagated on artificial media for some years while others were of quite recent isolation. Four strains were isolated from turkeys and two from arthritis in sheep.

Medium.—The basal medium used was a meat infusion made from either lean beef or horse meat as follows:

Meat infusion	500 cc.
Proteose peptone (bacto)	20 Gm.
Tryptone (bacto)	5 Gm.
NaCl	5 Gm.
Agar	15 Gm.
Water (distilled)	500 cc.

The infusion is made by placing 500 Gm. of ground meat with 500 cc. of distilled water overnight in the refrigerator. The mixture is then heated to 50° C. and pressed through cheesecloth, after which the fat is skimmed off. The other ingredients are dissolved in the water, heated to 50° C. and added to the infusion. The pH adjustment is made at pH 7.6 and the mixture is autoclaved for one hour at 120° C. The clot which forms is separated by filtration through cloth and the medium is then placed in flasks and sterilized. Before use the agar is melted and cooled and 10 per cent sterile horse serum is added before pouring into sterile tubes or plates. Broth of the same composition is made by omitting the agar. Nutrient gelatin is prepared by adding 12 per cent gelatin to the broth.

In fermentation studies serum was omitted from the broth. Sterilization was accomplished by dissolving the carbohydrates and alcohols in cold, sterile broth containing 1 per cent Andrade's indicator and filtering through Seitz or Berkefeld filters.

RESULTS

Morphology and Staining.—The morphology of the bacterial cells varied a great deal. When freshly isolated from mice, all strains formed colonies of the smooth type. The short, slender rods were straight, curved or comma-shaped. Paired cells were often noted. In young, actively growing cultures a characteristic arrangement of two straight rods forming an angle was observed. The short rods measured from 1 to 2 μ in length. Cultures that were repeatedly transferred showed a mixture of short rods and long, filamentous forms measuring 4 to 15 μ. Occasionally the long threads were bent and twisted into a mass resembling a mycelium. Branching was never observed.

The cells gradually lost their Gram-staining characteristic when cultured longer than six days. Many Gram-negative rods and filaments could then be seen. Beading of the larger forms could be observed by using Weigert's stain. The beaded appearance was more pronounced in cultures from broth than in those from a solid medium. In tissue or blood smears stained with Weigert's gentian violet the microörganisms could be readily detected singly or, more often, in groups of five to 50. Occasionally they were present in the phagocytes of the blood. In tissue or blood the bacterial cells were short, straight or curved rods. No metachromatic granules could be demonstrated. The strains studied were non-acid fast, non-spore-forming, and non-motile.

Cultural.—When tissue or blood of an infected animal was cultured, tiny round, shiny, transparent colonies about 1 mm. in diameter developed in 24 hours. They became opaque and gray in 48 hours, but did not increase in size. These smooth colonies were composed of short, slender rods. After repeated transfers the growth became visible in twelve hours as a light gray film. In 24 hours the growth was opaque, shiny and moist with a rough surface. The longer filamentous type of bacterial cell was found in this type of growth.

In serumized broth a faint turbidity developed in twelve hours which increased up to 24 hours. No pellicle was formed. A fine, gray sediment formed in 72 hours. The suspension in broth was quite stable. Cultures stood for 21 days with no clearing of the broth. Transfers made to broth

from rough colonies resulted in the formation of more sediment.

In gelatin-stab cultures incubated at 20° C., all strains developed the test-tube brush growth in 48 hours. In 24 hours colonies were seen along the needle track. These became larger and developed radiations from the center. After five days the growth reached the test-tube wall and on further incubation the typical appearance was lost. No liquefaction occurred in 21 days.

Surface colonies grew rapidly on blood-agar plates. In 24 hours the colonies were small, gray, round and firmly adherent to the medium. A narrow green zone of hemolysis developed around deep colonies in 24 hours. Complete hemolysis was not observed. No growth was visible on potato in 14 days.

Biochemic Characteristics.—No change in litmus milk was observed for four days. On the fifth day two cultures, one of swine origin and the other from a sheep, showed a slightly acid reaction. On the seventh day four more litmus milk cultures showed an acid reaction. Two of these were from swine and two from turkeys. Tests for indol formation, nitrate reduction, catalase formation, and methylene blue reduction were negative after four days of incubation. The Voges-Proskauer and methyl red tests were negative. No change in aesculin broth was observed in six days. A positive test for hydrogen sulfide formation was readily obtained in 24 hours in stab cultures on lead-acetate agar.

Gas was not produced on any of the fermentable media used. In the first series of tests, serum was added to the broth to accelerate growth. Positive reactions were then obtained on dextrose, sucrose, maltose, arabinose, xylose and dextrin in 24 hours. The control cultures, *Salmonella pullorum*, *Escherichia coli* and *Escherichia communior*, also gave positive results on these media. Serum was then omitted with the result that no reaction occurred in sucrose, maltose, arabinose, xylose or dextrin.

Of the 18 carbohydrates, two glucosides and seven polyhydric alcohols used, only four showed acid formation in 48 hours. These were dextrose, galactose, lactose and levulose. In the mannose broth all cultures were negative for two days when a faint reaction was seen in two cultures. Delayed reactions occurred in six more mannose cultures during the 21 days of incubation. The reaction in cellibiose was negative for nine days but in twelve days all but nine cultures showed acid formation. In 21 days all the cultures showed acid formation in cellibiose. None of the other fermentable materials gave positive tests for acid in three weeks.

All strains grew well aerobically and also under reduced oxygen tension. In culture tubes prepared by Wright's pyrogallic acid method the growth appeared in 24 to 48 hours as a shiny, gray, spreading growth. In stab cultures the growth was more abundant deep in the agar than near the surface.

After six days of incubation no growth appeared in broth at pH 6.0 to pH 6.4. A slight turbidity developed at pH 6.8 but at pH 7.2 to 7.6 growth appeared to be more abundant. At pH 8.2 a slight turbidity developed.

An interesting observation, not made a formal part of the study, was the resistance of the cultures to aging and drying. Twenty gelatin-stab cultures remained at room temperature during the summer for four months. Seventeen of them were revived when broth was added to the dried material. One set of cultures was kept at 50° C. for eight months in a refrigerator and found to be viable.

Pathogenicity.—All cultures were found to be pathogenic for mice. Death occurred in most cases in two to three days after an inoculation of 0.5 cc. of a 24-hour broth culture. A few mice survived for eleven days, but emulsions of spleens of these mice produced death in four days when reinoculated. Pigeons inoculated with either freshly isolated or old strains died within three days. Guinea pigs survived intraperitoneal injections of 1 cc. of 48-hour cultures.

SUMMARY

When isolated from inoculated mice, *Erysipelothrix rhusiopathiae* is a short,

slender, straight or curved rod 1 to 2 μ in length. On prolonged culture it forms long, filamentous threads from 4 to 15 μ long. The long, curved threads are often beaded. Both the short and the filamentous forms are Gram positive, but they decolorize readily on prolonged incubation.

Two types of colony formation are found. The short rods form small, round, shiny, discrete colonies with entire edges. The long, thread-like forms are found in colonies that are irregular in outline, have an uneven surface and are opaque in contrast to the translucent colonies of the short forms.

E. rhusiopathiae is not active in producing changes on commonly used media. In gelatin-stab cultures the growth radiates from the line of inoculation to give the appearance of a test-tube brush. A narrow zone of alpha hemolysis develops around deep colonies in blood agar. In litmus milk a slight acid reaction may be formed, but usually no change is observed. Potato does not support growth.

Indol is not formed and nitrates are not reduced. The Voges-Proskauer reaction, methyl red test, methylene blue reductase test and catalase test are all negative. Aesculin is not hydrolyzed. Lead-acetate medium is blackened by the formation of hydrogen sulfide.

The reaction on fermentable media is weak. No gas is formed. Acid is produced readily from only dextrose, lactose, galactose and levulose. A delayed reaction is seen on mannose and cellibiose. No acid formation is observed on arabinose, xylose, rhamnose, maltose, melibiose, sucrose, trehalose, raffinose, melezitose, dextrin, starch, inulin, amygdalin, salicin, glycerol, erythritol, adonitol, mannitol, sorbitol, dulcitol or inositol.

The microörganism will grow under reduced oxygen tension as well as anaerobically. An alkaline reaction, pH 7.2 to pH 7.6, is desirable for maximum growth. Mice and pigeons are susceptible to artificial inoculation. Guinea pigs are resistant. Virulence is increased by passage through susceptible animals.

References

[1]Pasteur, L., and Dumas, M.: Sur le rouget, ou mal rouge des porcs. Compt. rend. Acad. Sci., xcv (1882), pp. 1120-1121.

[2]Pasteur, L., and Thuillier, L.: La vaccination du rouget des porcs à l'aide du virus mortel atténué de cette maladie. Compt. rend. Acad. Sci., xcvii (1883), pp. 1163-1171.

[3]Loeffler, Dr.: Experimentelle untersuchung über schweine Rotlauf. Arb. a. d. k. Gsndhtsmte., i (1886), p. 48.

[4]Koch, R.: Untersuchungen über die Ätiologie der Wundinfektionskrankheiten (Leipzig, 1878). Gesammelte Werke von Robert Koch (Geo. Thieme, Leipzig, 1912), pp. 83-87.

[5]Löffler, F.: Zur Immunitätsfrag. Mitt. a. d. k. Gsndhtsmte., i (1881), pp. 134-187.

[6]Salmon, D. E.: An examination of Pasteur's vaccine for rouget. U.S.B.A.I. Ann. Rpt., ii (1886), pp. 187-194.

[7]Moore, V. A.: Mouse septicemia bacilli in a pig's spleen. J. Comp. Med. & Vet. Arch., xiii (1892), pp. 334-341.

[8]Smith, T.: Investigations of diseases of domesticated animals. U.S.B.A.I. Ann. Rpt., xiii and xiv (1897), pp. 166-174.

[9]Rosenbach, F. J.: Experimentelle morphologische und klinische Studien der erreger des Schweinerotlaufs, Erysipeloid and Mäuseseptikämie. Zeit. f. Hyg. u. Infekt., lxiii (1909), pp. 343-371.

[10]Prettner, Dr.: Ueber die Identität des Bacillus murisepticus und des Erysipelatos porci. Berl. Tierärztl. Wochnschr., xvii (1901), p. 669.

[11]Rickman, Dr.: Zur Frage der Erreger des Schweinerotlaufs, des Erysipeloid und der Mäuseseptikämie. Zeit. f. Hyg. u. Infekt., lxiv (1909), pp. 362-364.

[12]Rahm, H.: Zur Diagnose des Schweinerotlaufs. Med. Klin., xviii (1922), pp. 1217-1218.

[13]Zibert, S.: Zur Biologie der Schweinerotlauf bacillen. Tierärztl. Monatschr., ix (1922), p. 396.

[14]Zosel, O.: Ueber den microskopischen Aufbau der oberflächen und Tiefen kolonien des schweine Rotlaufbazillus auf Agar und Gelatine. Tierärztl. Monatschr., ix (1922), p. 408.

[15]Poels, J.: Rotlauf bei Tauben und Enten und Stammunterscheide bei Rotlaufbazillen. Folia Microbiol., v (1919), pp. 1-18.

[16]Eber, A.: Geflügel Rotlauf (Rotlauf-Septikämie der Vögel). Deut. Tierärztl. Wochnschr., xxiv (1921), pp. 295-298.

[17]Meyn, A.: Vergleichende Untersuchungen über Schweine und Geflügelrotlaufbakterien. Deut. Tierärztl. Wochnschr., xxxvi (1928), pp. 334-338.

[18]Schmidt - Hoensdorf, F.: Rotlauferkrankungen bei Vögeln im Anschluss an Schweinerotlauf und Mäuseseptikämie. Deut. Tierärztl. Wochnschr., xxxix (1931), pp. 196-197.

[19]Marsh, H.: The bacillus of swine erysipelas associated with arthritis in lambs. J.A.V.M.A., lxxviii (1931), pp. 57-63.

[20]Beaudette, F. R., and Hudson, C. B.: An outbreak of acute swine erysipelas infection in turkeys. J.A.V.M.A., lxxxviii (1936), pp. 475-488.

[21]Klauder, J. V., and Harkins, M. J.: Erysipeloid in the United States. J.A.M.A., xcvi (1937), p. 1205.

[22]Van Roekel, H., Bullis, K. L., and Clarke, M. K.: Erysipelas outbreak in turkey flocks. J.A.V.M.A., xcii (1938), pp. 403-418.

[23]Klauder, J. V., Righter, L., and Harkins, M. J.: A distinctive and severe form of erysipeloid among

fish handlers. Arch. Derm. & Syph., xiv (1926), p. 662.

[24]Kondo, S., and Sugimura, K.: Experimental studies on swine erysipelas bacillus found in fishes. J. Jap. Soc. Vet. Sci., xiv (1935), pp. 111-135.

[25]Schipp, C.: Zur Biologie des Schweinerotlaufbazillus und Zweier morphologisch gleicher Septikämieerreger. Deut. Tierärztl. Wochnschr., xviii (1910), pp. 97-101 and 113-117.

[26]TenBroeck, C.: Studies on *Bacillus muriseptic*us, or the rotlauf bacillus isolated from the swine in the United States. J. Exp. Med., xxxii (1920), pp. 331-343.

[27]*Ibid.*: Effect of enzymes in serum on carbohydrates and their relation to bacteriological technique. J. Exp. Med., xxxii (1920), pp. 345-349.

[28]Creech, G. T.: The bacillus of swine erysipelas isolated from urticarial lesions of swine in the United States. J.A.V.M.A., lix (1921), pp. 139-150.

[29]Meyn, A.: Über Glatt und Rauhformen bei Rotlaufbakterien. Cent. f. Bakt., Abt. I, Orig., cxxii (1931), pp. 507-510.

[30]Baker, D. W.: An account of the occurrence of hog erysipelas infection in New York state. Cornell Vet., xxiii (1933), pp. 66-70.

[31]Deem A. W., and Williams, C. L.: The fermentation reactions of *Erysipelothrix rhusiopathiae*. J. Bact., xxxii (1936), pp. 303-306.

[32]Giltner, L. T.: A fatal disease of young pigs apparently caused by the bacillus of swine erysipelas. J.A.V.M.A., lxi (1922), pp. 540-543.

[33]Cornell, R. L., and Glover, R. E.: Joint ill in lambs. Vet. Rec., v (1925), p. 833.

[34]Wayson, N. E.: An epizoötic among meadow mice in California caused by the bacillus of mouse septicemia or of swine erysipelas. Public Health Rpts., xlii (1927), pp. 1489-1493.

[35]Stiles, G., and Davis, C. L.: Swine erysipelas and its economic importance. J.A.V.M.A., lxxxiv (1934), pp. 895-906.

[36]Morrill, C. C.: Erysipeloid: occurrence among veterinary students. J. Inf. Dis., lxv (1939), pp. 322-324.

[37]Rosenwald, A. S., and Dickinson, E. M.: A report of swine erysipelas in turkeys. Cornell Vet., xxix (1939), pp. 61-67.

[38]Watts, P. S.: Studies on *Erysipelothrix rhusiopathiae*. J. Path. & Bact., l (1940), pp. 355-369.

[39]Barber, Mary: A comparative study of Listerella and Erysipelothrix. J. Path. & Bact., l (1939), pp. 11-23.

[40]Newsom, I. E., and Cross, F.: Some bipolar organisms found in pneumonia in sheep. J.A.V.M.A., lxxx (1932), pp. 711-719.

[41]Stuart, R. D.: Some observations on *Erysipelothrix rhusiopathiae* isolated from a human infection. J. Path. & Bact., xli (1935), pp. 369-370.

[42]Edwards, J. T.: A system of bacteriology. Med. Res. Counc., London, viii (1929), p. 379.

[43]Gustine, Dr.: Ausgeprägte Fadenbildung des Rotlaufbazillus in Tierkörper bei Endocarditis volvularis. Tierärztl. Wochnschr., vi (1912), pp. 97-98.

[44]Wyschelesky, S.: Bemerkenswerte Befunde Bezoglich des Wachstums des Bazillus des Schweinerotlaufs. Zeit. f. Infekt., xii (1912), pp. 43-53.

[45]Redlich, E.: Glatte und rauhe Kolonien bildende Rotlaufbakterien in Kranken Schweine. Zeit. f. Infekt., xlii (1932), p. 300.

[46]Schoening, H. W., Gochenour, W. S., and Grey, C. G.: Studies on *Erysipelothrix rhusiopathiae* with special reference to smooth and rough type cultures. J.A.V.M.A., xcii (1938), pp. 61-67.

[47]Sabella, A.: Involutionsformen des Bac. erysipelatos suis. Cent. f. Bakt., Abt. I, Orig., xciv (1925), p. 411.

[48]Kettlitz, Dr.: Die Ermittlung des Alkalitätsoptimum für Zuchtung des Rotlaufbacillus mit Hilfe des Komparatorverfahrens nach Michaelis. Arch. f. wiss. u. prakt. Tier., l (1924), pp. 334-340.

[49]Alphen, A. J. S. Van der: Bepalen der Voedingsboden reactie Bijulekziekte-culturen. Tijdschr. Diergeneesk., lvi (1929), pp. 232-235.

Microscopic Detection of Mastitis*

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I DO NOT WISH to leave the impression that a microscopic examination is all that is necessary in diagnosing mastitis, but it is a quick method of determining whether a cow is giving clean or infected milk. If many leucocytes are present, it is a sure sign that something is wrong and one must then seek for the cause of the trouble. Classifying streptococci and staphylococci bacteriologically is important and must be done, as Plastridge *et al*[1-3] have shown.

I have been concerned with detecting udder diseases microscopically and in checking my results with the pathologic findings in the tissues. If the samples of milk are carefully taken, it is surprising how few microörganisms will be found, unless one has a case of acute mastitis. If this mastitis milk is incubated, the organisms, which are no longer held back through immunologic processes, increase rapidly. Generally speaking, the more leucocytes there are, the fewer organisms there will be. Recently, Levaditi,[4-8] working on the abdominal exudate, coined the term "microscopically sterile" (fig. 1). This is what we have repeatedly found in milk and sometimes it was also sterile bacteriologically. On the other hand, microörganisms may be numerous and leucocytes scarce. Phagocytosis in the abdomen differs from that which we see in milk. In the former, the bacteria are finally destroyed by the mononuclear cells, but in the latter the polymorphonuclear leucocytes take the lead in ingesting and eliminating the organisms.

In describing pneumococcic infections, Levaditi, like many others, believes that the encapsulated forms of this organism are more virulent than the nonencapsulated cocci, and for this reason can not be phagocyted. He goes on to say that following physico-chemical changes, the cocci may be phagocyted, the visible effects being the disappearance of the capsule and the formation of a cytoplasmic vacuole in the leucocytes. My correspondence with Dr. Levaditi has unfortunately been interrupted; hence, it may not be possible to find out what he meant by physico-chemical changes or vacuole formation.* Evaluating the phagocytic changes in milk in terms of Levaditi's concept, one finds an obvious similarity, especially in the occurrence of clear areas surrounding the cocci, though this does not explain the whole process for milk, as the writer has not seen capsule formation. It is important to know how the microörganisms are destroyed and, especially, their microscopic appearance during the process in order to determine whether they are decreasing or increasing.

The first changes observed in the cocci were in cases where leucocytes were numerous and the cocci hard to find. They

*From the Ontario Research Foundation; presented as a lantern-slide demonstration before the Section on Research at the 77th annual meeting of the AVMA, Washington, D. C., August 26-30, 1940.

[1]Plastridge, W. N., Anderson, E. O., Weirether, F. J., and Johnson, R. E.: Infectious bovine mastitis. J. Dairy Sci., xix (1936), pp. 641-650.

[2]Plastridge, W. N., Anderson, E. O., and Seremet, J. S.: Infectious bovine mastitis. Bul. 224, Connecticut State Coll. (1938).

[3]Plastridge, W. N., Anderson, E. O., Williams, L. F., and Weirether, F. J.: Infectious bovine mastitis. Bul. 231, Connecticut State Coll., pp. 1-55.

[4]Levaditi, C., Vaisman, A., and Krassnoff, D.: Mécanisme de l'action chemiotherapique des dérivés à base de soufre dans les pneumococcies experimentales. Bul. Acad. Méd., cxix (1938), p. 553.

[5]Levaditi, C.: La chemiothérapie des infections microbiennes. Son mécanisme d'action. Zeit. Sch. Path. & Bact., i (1938), pp. 366-406.

[6]*Ibid.*: La chemiothérapie antiendotoxique. Ann. de l'Inst. Pasteur, lxi (1938), p. 635.

[7]Levaditi, C., Vaisman, A., and Krassnoff, D.: Mécanisme de l'action chemiothérapique de certains dérivés soufrés dans les pneumococcies experimentales. Ann. de l'Inst. Pasteur, lxii (1938), p. 36.

[8]*Ibid.*: Mécanisme de l'activité chemio de certains dérivés aromatiques sulfurés dans l'infection provoquée par le bacille de Friedlander. Bul. Acad. Méd., xxi (1939), p. 730.

*Since this address was given in Washington, I have seen some of Metchnikoff's early drawings of phagocytosis which show unstained rings surrounding the phagocyted bacteria. These appear to be identical to what I have called clear areas surrounding the cocci. Metchnikoff's illustrations were made available to me through the kindness of Professor W. L. Holman of Toronto University.

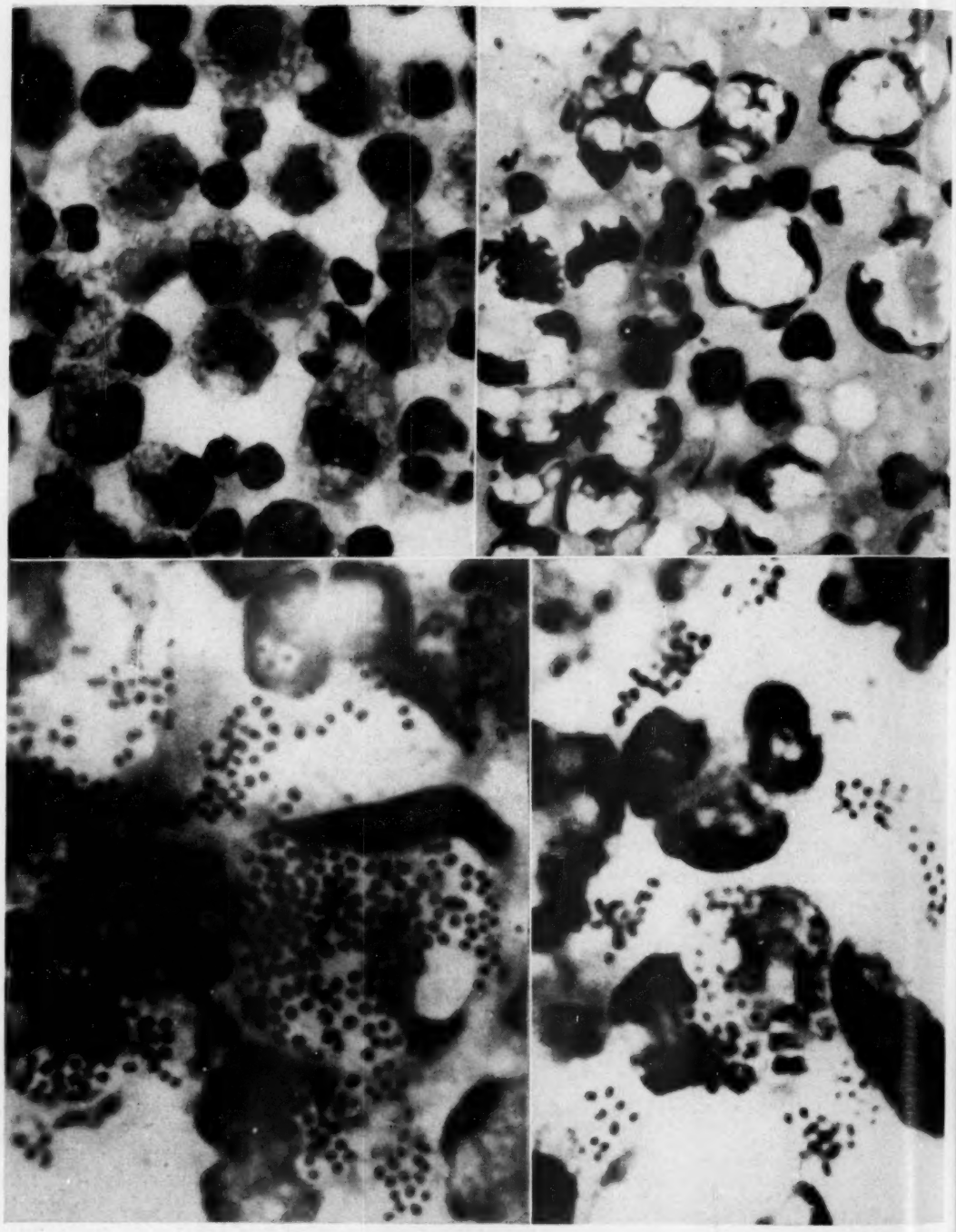

Plate I—Figs. 1-4

Left to right, top to bottom: (1) Large mononuclear leucocytes in microscopically sterile milk. Such milk also may be sterile culturally (x 86). (2) Polymorphonuclear leucocytes. The large cell on the right contains mucin; others are gorged on a colorless substance, forcing the nucleus over to the periphery of the cell (x 86). (3) General view of the cells in a severe case of staphylococcic mastitis; a few of the cocci can be seen with a halo of white surrounding them (x 2,900). (4) Phagocytosis. Dissolution of cocci, both outside and within the polymorphonuclear leucocytes. The cocci show a considerable decrease in size (x 2,900).

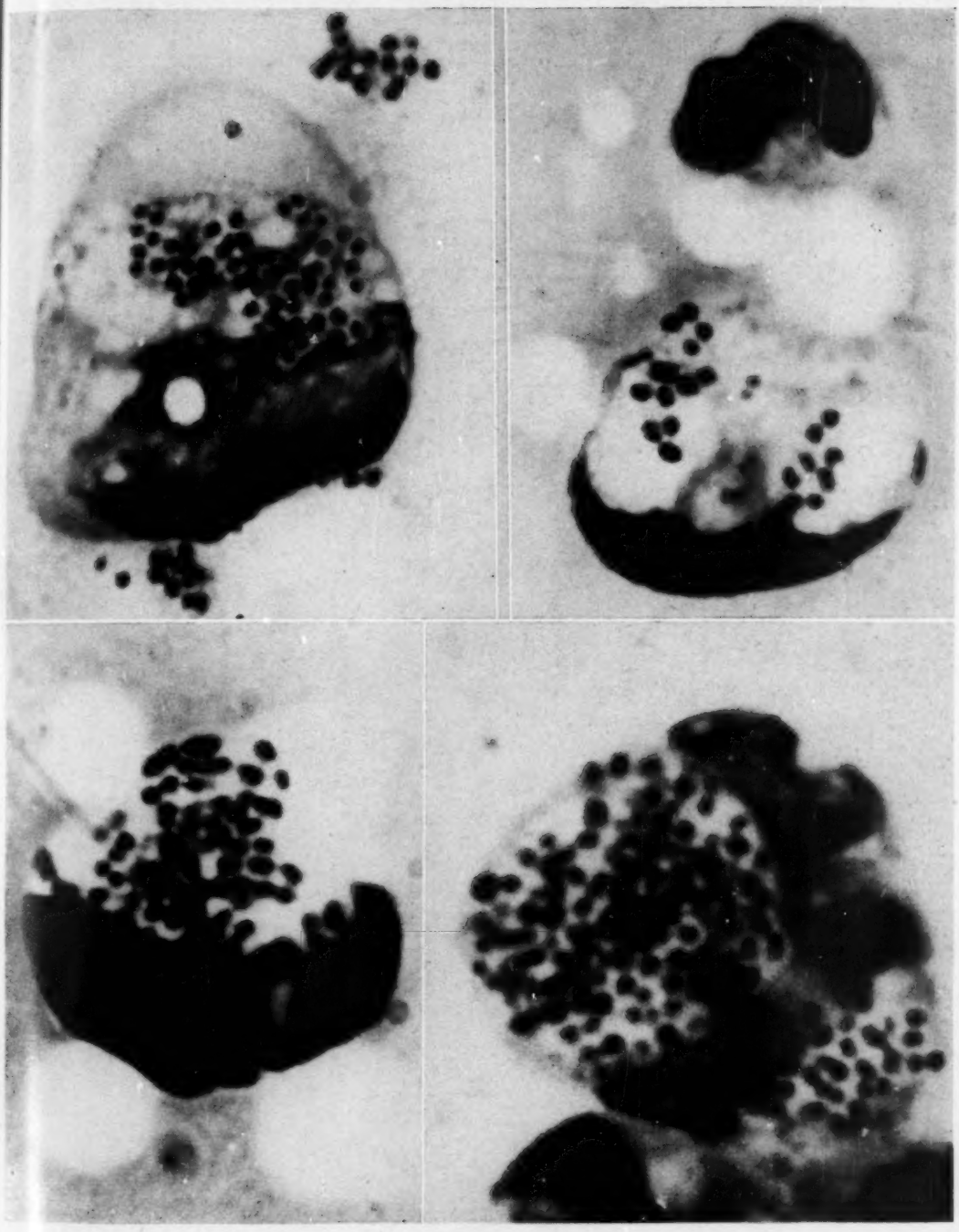

Plate II—Figs. 5-8

Left to right, top to bottom: (5) Early stage of phagocytosis and destruction of cocci. Some of the individual cocci stained a bright pink. They have an irregular appearance and a few are surrounded by a colorless halo (x 2,900). (6) Normal cocci in polymorphonuclear cell (x 2,900). (7) Irregular growth of cocci in leucocyte; a few of the individual cocci are disappearing (x 2,900). (8) Destruction of staphylococci in leucocyte. Note irregular size of cocci and white halo around cocci touching nucleus (x 2,900).

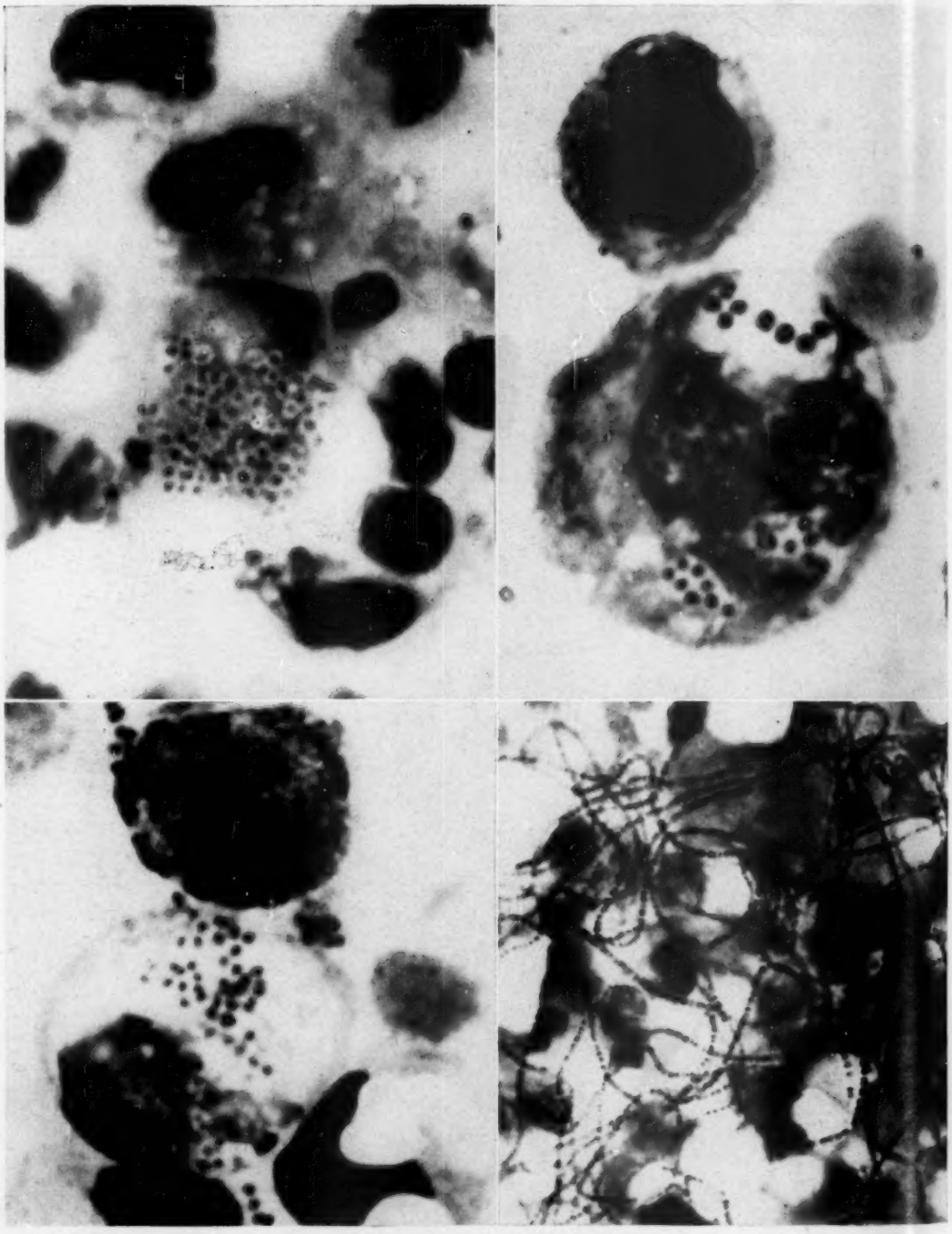

Plate III—Figs. 9-12

Left to right, top to bottom: (9) Note almost complete dissolution of cocci in leucocyte (x 2,900). (10) Large macrophage cell phagocyting cocci. The cocci in the clear area are normal in size. Those on the opposite side are disappearing (x 2,900). (11) Large macrophage cell ingesting cocci which are reaching the vanishing point. This is a comparatively rare occurrence, as the polymorphonuclears usually phagocyte the cocci (x 2,900). (12) Low power view of streptococci in milk sediment. The leucocytes are in a degenerative condition and the chains of cocci are actively growing (x 860).

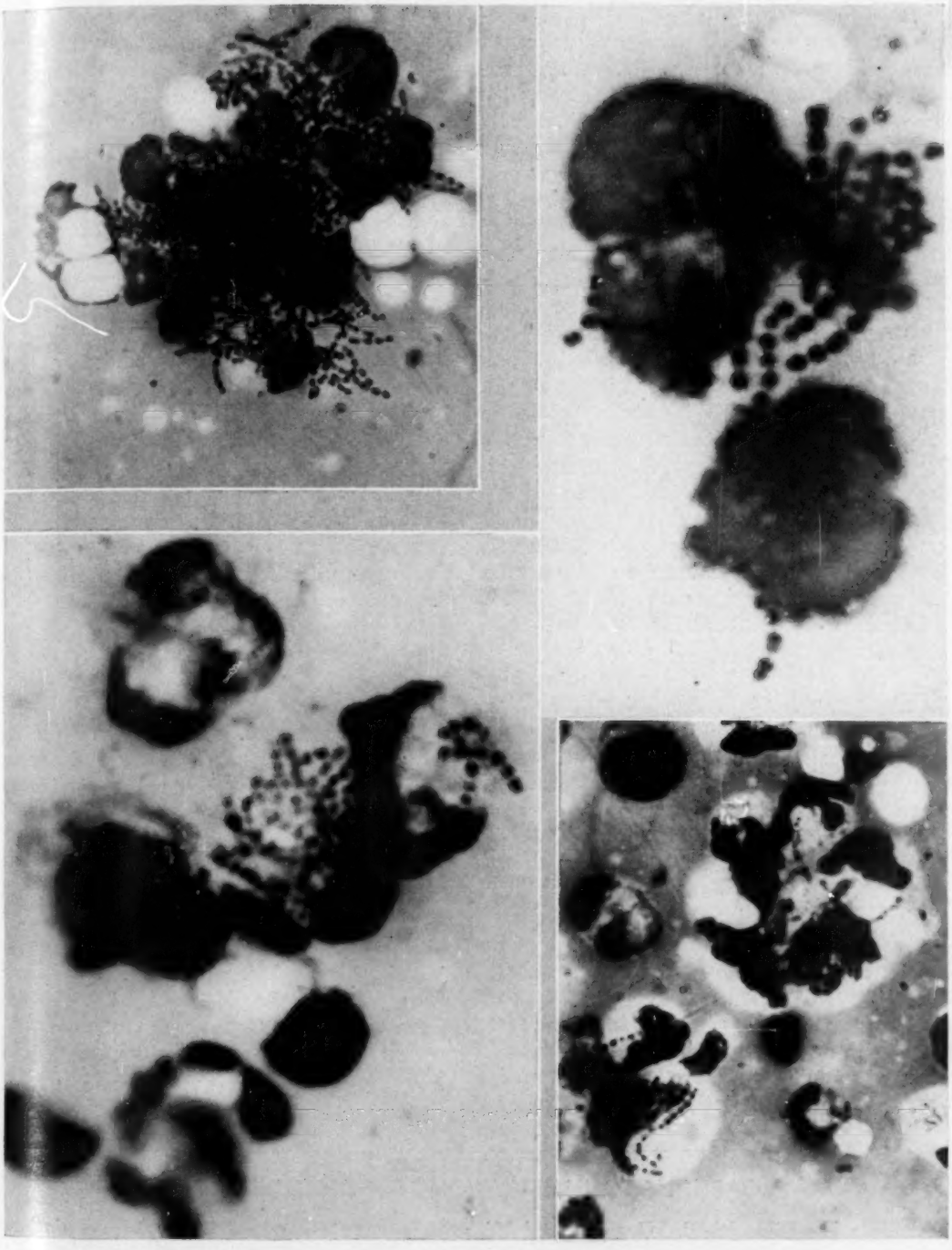

Plate IV—Figs. 13-16

Left to right, top to bottom: (13) A clump of streptococci being actively opposed by a group of leucocytes (x 860). (14) A group of streptococci on two lining cells from the udder (x 2,900). (15) A polymorphonuclear leucocyte attacking streptococci. Note degenerating chains on the left side and normal cocci on the other (x 2,900). (16) Active phagocytosis. The chains are being dissolved within the leucocytes (x 860).

were being phagocyted by the polymorphonuclear leucocytes. The cocci appeared fairly normal in size but irregularly arranged. Some of the cocci were colored a pinkish red with Hastings stain, which is different from their usual purplish-blue coloration and is possibly due to degenerative changes within the leucocytes (fig. 5). The most common changes in phagocyted cocci (figs. 7-9) are a diminution in size, and surrounding them a clear, unstained, circular area develops (fig. 3). The digestion of the cocci goes on until they are reduced to mere pin points (figs. 10-11). This may apply also to cocci which are not phagocyted (fig. 4). With streptococci, the effect of phagocytosis shows very clearly on the chains. Wherever they touch a leucocyte or lie across it, the grains disappear and a clear zone surrounds the chain on either side. The chains both inside and outside the leucocytes are sometimes destroyed in great numbers (figs. 15-16), making the process easier to detect than with staphylococci.

At this point I should like to refer to a paper I presented at the Chicago meeting of the AVMA in 1909 on the treatment of piroplasmosis. Professor Nuttall, with whom I was working at that time, had found that when an animal died of piroplasmosis, the parasites in the body rounded up and shortly afterwards disappeared. Thus, when he looked at my slides, which showed an increase in round forms following the injection of trypan blue, he said at once that the drug was killing them. This observation turned out to be correct and we had found a remedy for the disease. It is with a somewhat similar view that the present studies on milk have been made. There is a possibility that with a better understanding of phagocytosis in milk we may be able to gauge more accurately the curative effects on mastitis of many new drugs.

One point to be mentioned is the rapidity with which phagocytosis may occur. We have found that by taking samples at six-hour intervals, considerable changes are revealed. Recently, Munch-Petersen[9] said (regarding milk tests): "These results were based on fortnightly examinations. When the examinations were made more frequently, the streptococcus was found more often and in a larger number of cows." This may be the reason that chemical tests work well on one occasion but not on another—because day by day the udder reacts differently to infective organisms.

It is difficult to convince a dairyman of some of the above facts. Just recently, in two separate herds, three of the most active cases of staphylococcic mastitis happened to be giving an increased amount of milk. One of the animals, nevertheless, showed external evidence of inflammation. It is common knowledge that inflammatory processes may increase the natural secretions. In such cases one can not help wishing that milk did not have its white, concealing coloration and were penetrable to the naked eye.

During the past summer, I examined some milk in British Columbia and Alberta. In one herd of Jerseys which had been isolated for a number of years, I examined 15 cows and found only one quarter infected with staphylococci. Several of these cows had diphtheroids. In 16 beef cows there were four cases of staphylococcic in-

[9]Munch-Petersen: Studies on mastitis in dairy cows. J. Counc. Sci. Ind. Res., xiii (1940), pp. 19-23.

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

*Plate V—Figs. 17-23. Drawings of Milk Sediment Colored with Hastings Stain.**

(17) Polymorphonuclear leucocyte filled with mucinous material. A few mucin globules are nearby. (18) Normal polymorphonuclear cell. (19) Macrophage cell filled with fat droplets. (21) Nucleus of lining cell from the udder, with fat droplets. (21) Epithelial scales from teat canal with many diphtheroids. (22) Macrophage cell filled with leucocytes, mostly small mononuclears. (23) Lining cell nuclei from the udder in a case of Corynebacterium pyogenes infection.

*Drawings by Miss Peggy Clarke.

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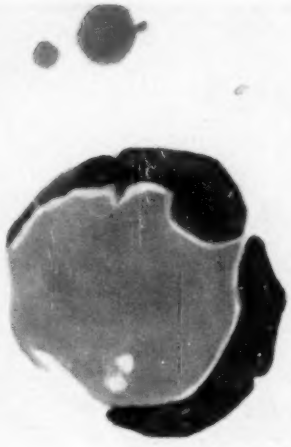

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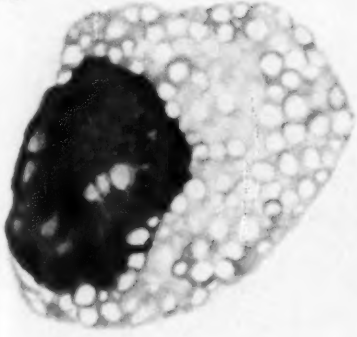

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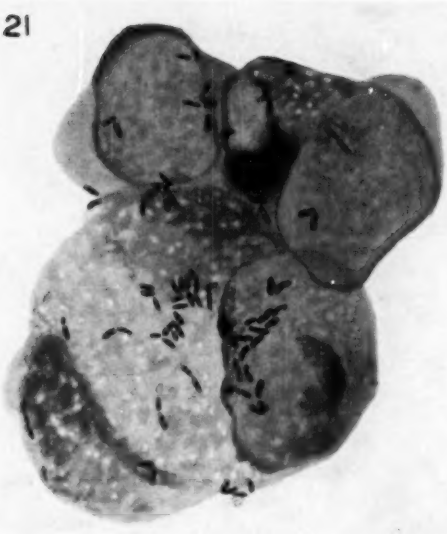

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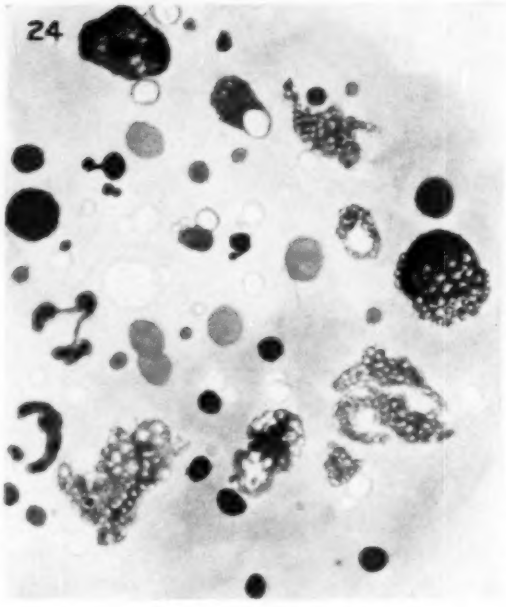
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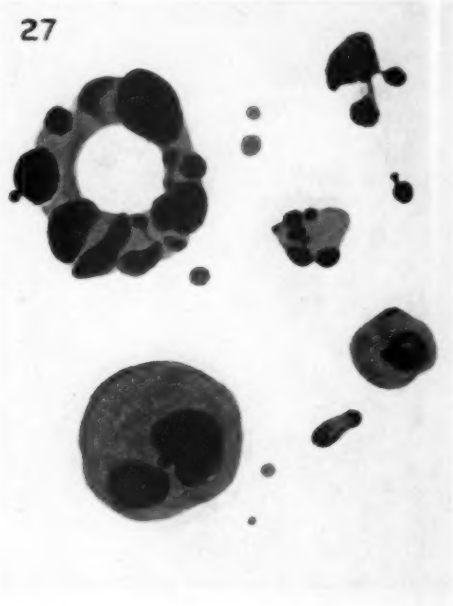
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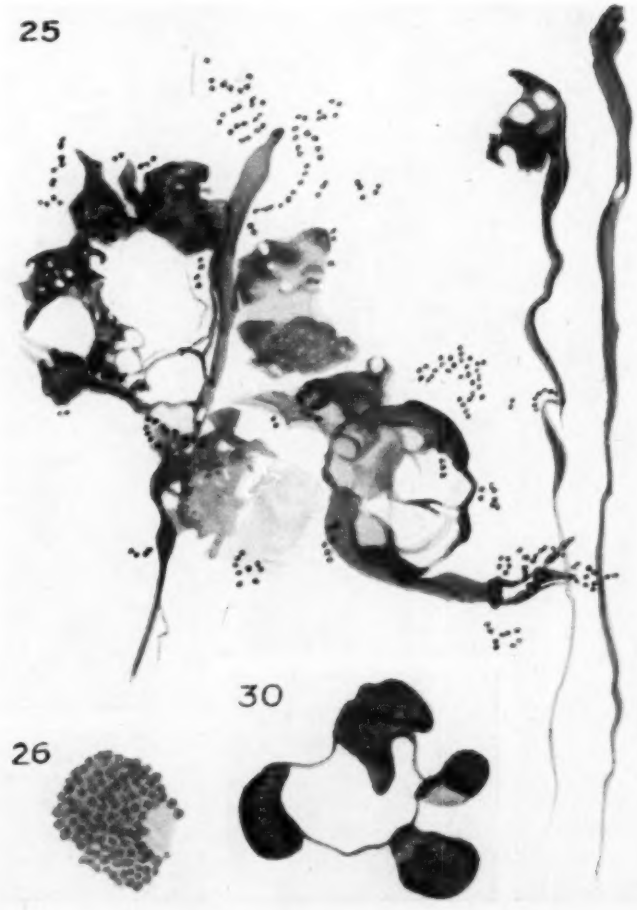
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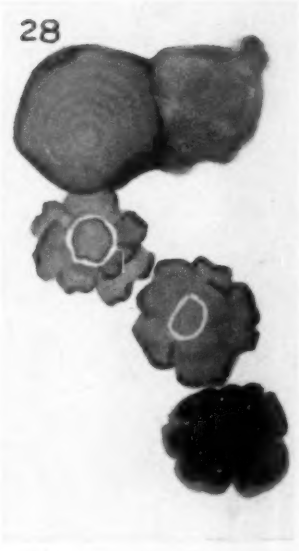
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fection, but in not a single case were diphtheroids found. Though diphtheroids are generally considered harmless, there is an impression in my mind that they may yet be proved to be forerunners of more serious trouble. Hopkirk[10] informs me that he has held this belief for some time. Munch-Petersen,[9] who has been experimenting on a herd of 50 isolated heifers, says: "With one exception, the herd was free from udder infections with corynebacteria."

On my trip into western Canada I was not able to obtain milk from range cows, as I had hoped. I had expected to find that range cows which only suckle their calves would show a low incidence of mastitis. However, in July I had the good fortune to secure five samples from reindeer cows at the mouth of the Mackenzie River. It was late in the milking season for reindeer, but they were all perfectly clean as far as the microscope could detect. In none did I find more than an occasional leucocyte.

I would like to make a few remarks on a case of actinomycosis which was recently diagnosed. A sample of pus was received at the laboratory from the jaw of a dairy cow which proved to be infected with actinomycosis. The following day the dairyman called up and said that he had forgotten to tell me that a lump in the udder was noted not long after the lesion on the jaw became prominent. Sections cut from the udder showed actinomycosis. Though I have heard little mention of the disease in this country [Canada] it is probably not rare in dairy cows since similar cases have been diagnosed and lesions of actinomycosis are commonly found during the inspection of meats.

I was recently asked to explain why leucocytes increase at the close of the lactation period. There is a similarity in the cell picture between colostrum and the last milk secreted. The probable reason for this is that there is retention of milk at this period, just as there is before lactation actually begins. I have referred to Porcher and Panisset,[11] who believe that colostrum is composed of the remains of milk previously secreted. It is evident that the nature of the milk and its cellular content will alter, inasmuch as in the process of drying up a cow, less and less milk is drawn from the udder.

Concerning the staining reactions obtained with Hastings stain, we have found that the selective action on certain cells, such as the lining cells, is helpful in recognizing them. It is for this reason that the two colored plates (figs. 17-30) are included in this paper, and it is believed that they will be of assistance to those who wish to use the microscopic method in the diagnosis of mastitis.

CONCLUSION

In concluding this account of the results of the microscopic diagnosis of mastitis, I should like to point out that the examination of milk sediment is quickly done and is useful as a preliminary method for classifying the cows in a herd. It has other advantages in that it is easy to repeat and requires little equipment other than the microscope.

[10]Hopkirk, C. S. M.: A method of control of mastitis in dairy cows. Australian Vet. J. (1933), pp. 53-61.

[11]Hadwen, S., and Gwatkin, R.: The detection of abnormal cow's milk by microscopic methods. Canadian J. Res. (1939), pp. 225-244.

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

*Plate VI—Figs. 24-30. Drawings of Milk Sediment Colored with Hastings Stain**

(24) Colostrum containing "blue bodies" which result from the breaking up of the mononuclear cells. (See figure 27.) Paler globules are composed of mucinous material. Cell debris and the nuclear remains of lining cells are seen. Red blood corpuscles are commonly observed. (25) Leucocytes breaking up. The polymorphonuclear cells form tangled skeins of fibrinous material. These are visible to the naked eye. (26) Eosinophile cell. These cells occur commonly on the smears, but in small numbers. (27) Break-up of mononuclear leucocytes. The nuclear fragments round up and form the "blue bodies" in colostrum. (28) Small calcium concretions found in the milk sediment. (29) Vacuoles in degenerating mononuclear cell. (30) Unraveling of polymorphonuclear leucocyte, early stage.

* Drawings by Miss Peggy Clarke.

Studies on Bovine Mastitis. Mastitis in Heifers*

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IN THE EXTENSIVE literature dealing with mastitis in the bovine species, very little has been reported regarding the disease in heifers.

Several workers have found mastitis streptococci in the milk of heifers immediately after calving. In the udder tissue of virgin heifers Hucker[1] reports that 47 per cent of the 21 virgin heifers and calves studied were found to harbor mastitis streptococci in their udders. In 1933, Minett, Stableforth and Edwards[2] found group II mastitis streptococci in the udder exudate of a heifer showing a swollen quarter just before calving. Klein *et al*,[3] in 1938, reported ten cases of mastitis occurring in one herd. These heifers developed unusual udder swellings in one or more quarters which were not hot or painful. "The first two series of samples collected revealed presence of beta hemolytic streptococci, of a type similar to *Str. agalactiae*, in the udders of all but three of the 10 heifers." This organism did not appear in any of the samples collected after the second set, either before or after calving, except in one heifer. They also report the presence of micrococci which, they state, persisted after the streptococcus disappeared from the secretions. "No attempt at classification of the micrococci was made, but the 'yellow,' hemolytic varieties predominated."

We have studied mastitis in heifers in three large herds of purebred dairy cattle. In one of these herds the disease assumed the form of an epizoötic which appeared first in the milking herd and later in the bred and unbred heifers. In the other two herds the disease has appeared sporadically in a few heifers. It is difficult, however, to estimate the frequency and importance of mastitis in heifers since the disease easily may be overlooked or no attention paid to slight swelling of the udder by the caretakers. Abnormal swellings of the udder in heifers are usually attributed to "sucking," or increased physiologic (hormone) activity. Many of the heifers which show abnormal swelling of the udder as yearlings or 2-year-olds freshen as normal milk producers. This will be true if the swelling was due to increased physiologic activity. Such cases appear to be quite common, but they do not constitute true mastitis. If the swelling is caused by a low-grade infection, the heifer may be fully recovered by the time she freshens, or although there is little or no physical change in the udder structure and the milk appears normal, the infecting microörganism may be present in the milk. If, however, the swelling is caused by microörganisms of high virulence, serious damage will result to the udder. Such heifers may freshen with "blind" or partly "blind" or "slack" quarters, and the milk is often abnormal in physical and chemical properties.

Clinical History

HERD ONE

This is a herd of approximately 150 purebred Holstein-Friesian cows and heifers. The herd has been established about 25 years, and during the past 20 years no females have been added. Until the summer of 1938, mastitis was not a problem in this herd. In August 1938, mastitis appeared suddenly in several of the milking cows. The infected animals presented symptoms of acute mastitis involving one or more quarters of the udder. The cows were milked by hand twice daily and were on pasture during the day. On August 28, 1938, the cows were apparently normal at the morning milking, but at the evening milking several of them refused feed, they had chills and elevated body temperatures of one or two degrees, and several had diar-

*A project of the Haskell Animal Disease Research; from the Department of Bacteriology and Hygiene, University of Delaware. Presented before the Section on Research at the 77th annual meeting of the AVMA, Washington, D. C., August 26-30, 1940.

[1]Hucker, G. J.: Mastitis. V. The presence of mastitis streptococci in bovine mammary tissue. New York State Agr. Exp. Sta. Tech. Bul. 241 (1937).

[2]Minett, F. C., Stableforth, A. W., and Edwards, S. J.: J. Comp. Path. & Therap., xlvi (1933), p. 131. Cited by Klein *et al*.[3]

[3]Klein, L. A., Kleckner, A. L., and Scheidy, S. F.: *Str. agalactiae* infection in heifers prior to parturition. Univ. Pennsylvania Bul., Vet. Ext. Quar., xxxviii (1938), pp. 3-9.

rhea. There was pronounced agalactia and inflammation in one or more quarters of the udder. The milk from the involved quarters was abnormal. It contained clumps of casein, and in some of the animals it was the color of coffee. All of the mature cows made rapid, spontaneous recoveries without treatment. The blood plates revealed infection with a wide-zone hemolytic *Staphylococcus aureus*. The milk filtrate revealed a bacteriophage giving 2+ or 3+ lysis against the specific organism carried in the milk. The blood serum of the infected animals eventually agglutinated the organism in dilutions of 1:100 and above.

The majority of the heifers which became infected suffered serious damage to the udder, except those with nonfunctioning udders which were treated early. If the infection did not occur until after calving, the damage to the udder was considerably lessened.

Heifer 404 was a case of this type. This animal developed mastitis in the right fore quarter a few days after freshening with her first calf. There was no systemic reaction, but the involved quarter was inflamed with the milk of this quarter separated into whey and coagulated solids and greatly reduced in amount. Ox-blood-agar plates of this milk showed many colonies of double-zone hemolytic *S. aureus* with a very large zone of hemolysis (table I, No. 404). Also on the plates were *Escherichia coli* and a few nonhemolytic streptococci. The blood serum of this animal eventually gave a positive agglutination against *S. aureus* antigen in a titer up to and including 1:1,600. She was treated by injecting into the udder *via* the teat duct bacteriophage specific for the *S. aureus* carried in the milk. Her milk was negative for natural bacteriophage. The bacteriophage was injected at intervals of one week. The milk from this quarter soon became normal in appearance, but was lowered in amount approximately 80 per cent compared with the normal quarters. The staphylococci continued to be present for several weeks.

The heifers which became infected during their first pregnancy suffered severe damage to the udder, except those which were treated early.

Heifer 433, which freshened with a "blind" left rear quarter, was an example of this. This animal, when pregnant three months with her first calf, developed a swollen left rear quarter. The case did not come to our attention at the time, and the only treatment given consisted of stripping the infected quarter. Under this treatment the quarter appeared to improve and return to normal. The swelling subsided and the liquid exudate was no longer present. When she freshened, the involved quarter was filled with milk, but it was impossible to remove the milk *via* the teat. The remaining quarters milked normally. The heifer was slaughtered and the udder obtained for study. This study revealed three normal quarters. The left rear ("blind") quarter revealed that the lactiferous sinus and duct were completely obliterated by proliferated connective tissue. The duct was completely closed. The alveoli, although filled with milk, appeared to have undergone atrophy, and there was an excessive proliferation of connective tissue throughout the interlobular spaces.

Heifer 441 was observed to have a swollen left fore quarter about three weeks prior to calving. She was first seen by one of us on May 19, 1939, a few days prior to calving. At this time palpation of the udder was difficult because of the congestion and edema which were present. The swollen quarter contained a thick, pus-like exudate which on bacteriologic examination revealed a hemolytic *S. aureus* and a few nonhemolytic streptococci. The right fore quarter appeared normal except for the teat duct which was closed. Both rear quarters and teats were normal. The heifer calved on May 22, 1939. Examination after calving revealed the left fore quarter to be filled with milk, but only a small amount of thick, pus-like exudate could be removed from the teat. This material when streaked on ox-blood agar gave a pure culture of a hemolytic *S. aureus* (table I, No. 441). The right fore quarter appeared normal except for the occluded teat, which made it impossible to remove any of the milk. Both rear quarters milked normally and gave normal milk. Examination on June 2 revealed increased induration, especially at the base of the teats of both fore quarters. The heifer was slaughtered and the udder obtained for study. Gross examination revealed normal rear quarters. The left fore quarter showed a large amount of proliferated connective tissue around the wall of the lactiferous sinus. The duct appeared normal. A thick, tenacious mucoid exudate was present in the sinus and in the duct. In the right fore quarter the lactiferous sinus and duct were completely obliterated by proliferated connective tissue. There was also an excessive amount of proliferated connective tissue throughout the interlobular spaces.

Following the above experiences, all heifers showing abnormal swellings of the udder were examined immediately and the secretions tested bacteriologically. In 1939, there were 31 heifers 12 to 20 months of age on this farm, running together in the

same pasture. They had never been in contact with the milk herd. Early in the summer two of the heifers developed clinical mastitis at about the same time and, later, four others developed the disease. Several others were undoubtedly infected but did not show clinical symptoms of the disease. This is indicated by the fact that two heifers without clinical history of mastitis freshened with "blind" quarters.

The six heifers which developed clinical mastitis were treated by injecting bacteriophage specific for the infection into the lactiferous sinus *via* the teat duct. Five of the heifers apparently have made complete recoveries. One heifer developed multiple abscess formation in the infected quarter. Four of these heifers were infected with *S. aureus* (table I, Nos. 477, 478, 480 and 449).

Heifer 449 is typical for this group. In July, when pregnant 3½ months, she was observed to have a swollen right fore quarter. A sample of the exudate from this quarter was brought to the laboratory on July 15, 1939. The bacteriologic examination revealed a pure culture of *S. aureus*. This organism was completely lysed with our polyvalent *S. aureus* bacteriophage. On July 21, this heifer was examined by one of us. The right fore quarter was swollen and hard and there was an edematous swelling in front of the quarter. The remaining quarters were normal on palpation. Samples of exudate were collected from each quarter. From the right rear quarter 10 cc. of a light-gray secretion was obtained. This secretion coagulated into a firm clot within a few minutes after collection. The right fore (swollen quarter) gave a grayish-colored exudate containing flocculi and it did not coagulate. The two left quarters gave a light gray secretion; there were no flocculi, but it did not coagulate. Based upon this evidence of failure to coagulate, although only the right fore quarter was swollen, a clinical diagnosis was made of infection in three quarters—namely, the swollen quarter or right fore, and the two left quarters. The blood-plate test confirmed this diagnosis. The right rear quarter was sterile. The others contained hemolytic *S. aureus*. These staphylococci from the three infected quarters were lysable with our stock staphylococcus bacteriophage. Five cc. of bacteriophage was administered into each quarter on July 25, 26, 28 and 31. Examination on July 31 revealed the swelling in the right fore quarter had subsided and the bacteriologic examination of the small amount of secretion present in each quarter revealed no growth. There was no recurrence of the mastitis throughout the remainder of pregnancy. The heifer freshened on December 4, 1939. She has milked normally in all quarters, and her milk was normal on bacteriologic test.

In December 1939, one heifer in this group and in March 1940, another heifer in the group developed clinical mastitis with an entirely different bacteriologic picture. The exudate from the udders of these heifers gave pure cultures of an unusual streptococcus which will be described later.

On December 19, heifer 482 was discovered to have a swollen left rear quarter. The swollen quarter was hard, about the size of a baseball and contained a thick, pus-like exudate. The blood plate revealed a pure culture of a streptococcus producing minute, pin-point, slow-growing alpha hemolytic colonies. The infected quarter was treated by daily injections *via* the teat duct of bacteriophage specific for this organism. The swelling rapidly subsided and on January 9, and 15, the exudate from this quarter was sterile on bacteriologic examination. On March 5, 1940, she was examined for pregnancy and pronounced pregnant. The left rear quarter on this date appeared normal on palpation. The teat duct appeared somewhat thickened.

On March 15, 1940, heifer 479 was discovered to have a swollen right fore quarter. From this quarter 3 to 4 ounces of a fetid, pus-like exudate was obtained. Ten cc. of polyvalent mixed bacteriophage was injected into the sinus of the infected quarter. This was followed by a severe reaction as indicated by increased swelling of the infected quarter and elevation of body temperature to 105° F. In the majority of heifers previously treated with bacteriophage, no reactions occurred. In a few heifers the injection was followed by mild swelling of the udder which rapidly subsided. This heifer developed a marked systemic reaction in which the temperature remained at the 105° F. level for several days. The bacteriophage injections were continued. The exudate from the infected quarter gave a pure culture of slow-growing, minute, pin-point alpha hemolytic streptococcus colonies. The streptococcus was lysable with our stock streptococcic bacteriophage and treatment was continued with bacteriophage specific for her infection. The swelling receded very slowly and although the exudate was decreased in amount, the streptococcus continued to be present. By April 4, it became apparent that multiple abscesses were forming in the infected quarters. On April 10, the heifer aborted a 7-month-old fetus. The udder healed promptly following drainage of the abscesses. Because of the loss of the fetus and the damaged quarter, the heifer was

slaughtered. The streptococcus isolated from this heifer was injected into one quarter of the udder of a yearling grade heifer. This animal developed a typical local reaction. The artificially infected quarter became swollen to about the size of a baseball. The swelling was hard, hot and painful. There was no systemic reaction or abscess formation. No treatment was given and the swelling subsided in about three weeks. The sinus appeared to be filled with connective tissue.

In the 1940 crop of yearling heifers on this farm, one case of mastitis has been found to date. These heifers have not been in contact with the remainder of the herd.

On May 13, 1940, while examining the udders of this group of heifers for supernumerary teats, heifer 517 was observed to have a swollen right rear quarter. The swelling was soft and nonpainful. The teat orifice appeared to be well sealed. This quarter contained a straw-colored exudate which did not coagulate. The left rear quarter was slightly swollen and contained exudate. The exudate from the right rear quarter gave a pure culture of a hemolytic *S. aureus*. The exudate from the left rear quarter was sterile. On May 17, the heifer received 10 cc. of bacteriophage specific for her infection in each rear quarter. Before the bacteriophage was injected, the exudate was removed. This again showed *S. aureus* on the right side and sterile on the left side. Treatment was repeated on May 19. Samples of the exudate taken on May 20 and 21 were sterile. On May 29, her caretaker, without our knowledge, injected bacteriophage into the rear quarters. This was followed in a few hours by a pronounced edematous swelling of the injected quarters. We saw the heifer during the height of the reaction. Samples of the exudate were collected and when tested bacteriologically were found to be sterile. The swelling subsided rapidly and 24 hours later the udder appeared normal.

The total number of clinical cases of mastitis which have been studied in the heifers of this herd up to the time of writing is 18 (table II). Five of the heifers were recently fresh, three were in advanced pregnancy and near calving ("springing"), five were 2-year-old pregnant heifers, and five were yearling heifers. In addition, there were two infected heifers which were either overlooked or which did not show clinical symptoms of the disease. This is indicated by the presence of "blind" or damaged quarters at freshening (table II, Nos. 466 and 472).

HERD TWO

This is a herd of purebred Guernsey cattle of approximately 130 cows and heifers.

During the summer of 1938, two yearling heifers which were on pasture with about 20 others of similar age were observed to have swollen udders. When these heifers were examined by one of us, the infection was found to involve two quarters in one of the heifers and three quarters in the other heifer. These swellings were hard, hot and painful. From the teats of the involved quarters a small quantity of pus-like exudate could be removed. Bacteriologic examination revealed pure cultures of *S. aureus*. Treatment consisted of warm fomentations and frequent stripping. They made very slow recoveries, the involved quarters continuing to be hard and indurated. The heifers were bred and were sold for slaughter soon after freshening because of bad quarters. We did not see these animals after they calved, but we were informed that all of the involved quarters were either "blind" or gave "bad" milk.

In the older bred heifers one case developed.

This heifer was observed to have a swollen right rear quarter during early pregnancy but was not treated. When she freshened the left side of the udder was normal, but both quarters on the right side gave "bad" milk. She was seen by one of us one week after calving. The secretion from the involved quarters was purulent, fetid and contained strings of pyogenic membrane. A quart or more of this material could be removed from each involved quarter. A darkened (black) area the size of a silver dollar was noted on the posterior part of the right rear quarter. Three days later the right rear quarter ruptured at the darkened area. As necrosis was well established in both right quarters, the heifer was destroyed. The bacteriologic examination revealed in the right rear quarter a pure culture of a streptococcus which produced very slow-growing, minute, pinpoint colonies giving alpha hemolysis. The right fore quarter gave a similar streptococcus and a hemolytic *S. aureus*.

In 1939, only one case developed in the heifers of this herd.

This animal, an 18-month-old heifer, was on pasture with 20 others of similar age. The herd was inspected daily and there was no history of sucking. In June, this heifer was observed to have a swollen right rear quarter with exudate escaping from the teat. The exudate when examined was sero-caseous with relatively large amounts of straw-colored serum which did not clot on standing. On bacteriologic examination this quarter gave a pure

TABLE I—Staphylococcus Aureus Isolated from Heifers with Clinical Mastitis

Animal or Culture	Hemolysis		Ox-Blood Plasma Coagulation	Rabbit-Blood Plasma Coagulation	Gelatin Lique.	Crystal-Violet Agar	Mannitol	Litmus Milk			Bacteriophage Action	Rabbit-Skin Test	Clinical History
	Ox-Blood Plate	Ox-Blood Tryptose Broth						Acid	Reduction	Curd			
B-1	+	+	+	+	+	—	+	+	—	+	4+	2+	Acute mastitis, 3 quarters infected; staphylococcemia; died, 3d day
B-2	+	+		+	—	V*	+	+	—	—	4+	3+	Acute mastitis, 3 quarters infected; staphylococcemia; died, 10th day
404 (1)	+	+	+	+	+	V	+	+	+	+	4+	2+	Acute mastitis, 1 quarter infected; no systemic reaction; slow recovery
441 (2)	+	+	—	+	—	—	+	+	—	—	4+	4+ (3)	Acute mastitis; rapid fibrosis; two "blind" quarters
477	+	+	+	+	+	V	+	+	+	+	4+	4+	Acute mastitis, 1 quarter infected; no systemic reaction; recovered
478	—	—	—	+	—	—	+	+	+	+	4+	2+	Acute mastitis, 3 quarters infected; no systemic reaction; recovered
480	+	—	—	+	+	—	—	+	+	+	4+	— (4)	Acute mastitis, 2 quarters infected; no systemic reaction; recovered
449	+	+	—	+	+	V	+	+	+	+	4+	2+	Acute mastitis, 3 quarters infected; no systemic reaction; recovered
446	—	—	—	—	—	—	—	+	—	—	4+	2+	Acute mastitis, 2 quarters infected; systemic reaction; necrosis of udder
406	+	—	—	+	—	V	+	+	—	—	4+	2+	Acute mastitis, 4 quarters infected; no systemic reaction; recovered
417 (5)	+	+	—	+	—	—	—	+	+	+	4+	1+	Acute mastitis, 4 quarters infected; no systemic reaction; recovered
L-3	+	—	—	—	—	—	—	+	+	—	4+	1+	Acute mastitis, 2 quarters infected; systemic reaction; necrosis of udder
L-4	+	—	—	—	+	—	—	+	+	—	4+	1+	Acute mastitis, 4 quarters infected; no systemic reaction; recovered
517	+	—		—	—	V	—	+	—	—	4+	2+	Acute mastitis, 1 quarter infected; no systemic reaction; recovered

V = Violet. (1) This culture killed mice in 5 hours, 45 minutes, 0.5 cc. of 24-hour broth cultures being used; (2) alpha hemolysis; (3) diffuse inflamed area 6 cm., with 4-cm. necrotic area; (4) this culture was old when tested on rabbit; (5) this culture killed white mice in 22 hours, 0.5 cc. of 24-hour broth culture being used.

culture of a hemolytic *S. aureus*. The remaining quarters, although not swollen, gave a mixture of hemolytic and nonhemolytic colonies of *S. aureus*. The swollen quarter contained an area of fibrosis about the size of a golf ball located high in the posterior part of the quarter. Treatment consisting of injecting 2.5 cc. of polyvalent *S. aureus* bacteriophage, which gave 4+ lysis against her organism, into each quarter was started on June 16, and repeated daily for ten days. The blood plate of her exudate was sterile on June 21, but treatment was continued until the 26th. On July 3, a check-up on this animal revealed a few nonhemolytic staphylococci which were considered to be normal flora. Some of these colonies were moth-eaten and bacteriophage specific for her organism was isolated from the blood plate by touching the moth-eaten area of a colony. As the area of induration was still present and there appeared to be some induration at the base of the right rear teat, another course of treatment with bacteriophage was given. Examination on July 18 revealed the quarter to be much improved. On June 15, the blood serum of this heifer was negative for *S. aureus* agglutinins. On September 14, her blood serum gave a positive agglutination reaction in 1:100 dilution and partial agglutination in the 1:200 dilution. This heifer freshened in March 1940. Her udder was normal on physical examination and when fresh ten days she was producing 35 lb. of milk daily. Her milk was negative for *S. aureus*.

HERD THREE

This is a herd of purebred Guernsey cattle of approximately 200 cows and heifers.

Two cases of mastitis in heifers have been studied in this herd.

In September 1939, a mature cow in this herd developed acute mastitis involving three quarters of the udder two days following a normal parturition. The infection rapidly became generalized (bacteremia), the body temperature being elevated to 107.2° F. The animal received neutral acriflavine, 1 Gm. in 500 cc. of physiologic saline, intravenously following which the temperature dropped to 103.8° F. She continued to show pronounced symptoms of toxemia and the temperature in 24 hours returned to 105° F. The neutral acriflavine was repeated, but with little or no benefit, and death occurred on the third day. Each day of her illness a hemolytic *S. aureus* (table I, B1) was isolated from three quarters of the udder and from the blood stream in pure culture.

In March 1940, a heifer in this herd freshening with her first calf developed acute mastitis with bacteremia. Her case was nearly identical with the one just described. This heifer calved on March 6, 1940. She was normal for 48 hours when she developed an inflamed right rear quarter. Her temperature became elevated to 104.9° F. When seen by one of us a few hours later, the temperature was 105.8° F. Milk samples were collected from each quarter and 30 cc. of polyvalent *S. aureus* bacteriophage was administered intravenously. The right rear quarter (inflamed quarter) gave large numbers of a pure culture of a hemolytic *S. aureus* (table I, B2). The right fore quarter was sterile and the two left quarters gave numerous hemolytic *S. aureus*. The blood culture from the external abdominal vein gave a pure culture of a hemolytic *S. aureus*. A diagnosis was made of *S. aureus* mastitis with

TABLE II—Clinical History of Heifers with Mastitis on Farms 1, 2 and 3

Age of Heifer and State of Lactation	No. of Animal	Type of Mastitis	Treatment and Termination of Case
Farm 1			
2½-3 years; recently fresh	417	Acute	Not treated; lost 50% of infected quarter
	421	Acute	Not treated; complete recovery; had natural bacteriophage in milk
	404	Acute	Treated with bacteriophage; lost 80% of infected quarter; normal udder and calf
	406	Acute	Treated with bacteriophage; complete recovery
	410	Acute	Treated with bacteriophage; complete recovery
2½-3 years; about to freshen, "springing"	426	Acute	Treated with bacteriophage; became dry in infected quarter; this quarter "blind" when fresh with 2d calf
	446	Acute gangrenous	Treated with bacteriophage; infected quarters became necrotic; slaughtered
	441	Acute	Not treated; two quarters "blind" when fresh
2 years; pregnant few months	418	Acute	Not treated; "blind" quarter when fresh
	423	Acute	Not treated; lost infected quarter when fresh
	433	Acute	Not treated; one quarter "blind" when fresh
	449	Acute	Treated with bacteriophage; complete recovery; normal in all quarters when fresh
	466	No symptoms	Freshened with "blind" quarters; no treatment
	472	No symptoms	Freshened with "blind" quarters; no treatment
	479	Acute with abscess	Treated with bacteriophage; marked systemic reaction; abscess formation; aborted 7-month fetus
1 year	477	Acute	Treated with bacteriophage; apparently recovered; due to calve November 1940
	478	Acute	Treated with bacteriophage; apparently recovered
	480	Acute	Treated with bacteriophage; apparently recovered; due to calve October 1940
	482	Subacute	Treated with bacteriophage; apparently recovered; due to calve September 1940
	517	Subacute	Treated with bacteriophage; apparently recovered; not bred
Farm 2			
2 years	L-3	Acute	No treatment; when fresh, infected quarters became necrotic; slaughtered
1 year	L-1	Acute	Treated by stripping; "blind" quarters when fresh
	L-2	Acute	Treated by stripping; "blind" quarters when fresh
	L-4	Acute	Treated with bacteriophage; complete recovery; normal udder when fresh
Farm 3			
2½ years; fresh	B-2	Acute with staphylococcemia	Treated with bacteriophage intravenously; blood became sterile on 7th day; udder not improved; died of pneumonia 10th day
1 year	B-3	Subacute	Treated by stripping; history not complete; not bred

staphylococcemia. Three quarters of the udder were infected, although clinically only one quarter appeared to be involved. As the *S. aureus* bacteriophage appeared to be well tolerated by the animal, it was decided to continue this line of treatment. Six hours after the first dose was given, an additional 50 cc. of bacteriophage was administered intravenously.

On March 9, the heifer received 50 cc. of bacteriophage. Her temperature was 105.2° F., but she appeared slightly improved. By this time we had checked our stock bacteriophage against her specific staphylococcus and found that it gave 4+ lysis. A large quantity of specific bacteriophage was prepared and administered intravenously as follows: March 10, 100 cc.; March 11, 100 cc.; March 12, 50 cc.; March 13, 100 cc.; March 14, 100 cc.; March 15, 175 cc.; March 16, 100 cc.; and March 17, 100 cc. The temperature remained at the 105° F. level until March 15, when it dropped to 103.8° F. at both a. m. and p. m. readings. On March 16, the temperature was 102.6° F. On March 17, it was 102° F.

Daily bacteriologic examinations were made of the milk and blood. The blood stream continued to show *S. aureus* until March 15, when it was sterile and remained so. The right fore quarter remained normal until March 12, when the milk from this quarter gave numerous minute, pin-point colonies of a nonhemolytic streptococcus. On the 13th, this organism was present in two quarters and on the 14th it appeared in the blood stream. This streptococcus was lysed by our polyvalent streptococcus bacteriophage and after this date bacteriophage specific for this organism was added to the staphylococcus bacteriophage which the heifer received. Until the termination of the case both microörganisms continued to be present in the milk.

On the 15th, the heifer appeared improved. With the temperature down to 102.6° F. on the 16th, and 102° F. on the 17th, her recovery appeared promising, although there was little or no improvement in the mammary gland. On the 18th, the animal showed symptoms of pneumonia and died on that date. The autopsy confirmed the diagnosis of pneumonia. The udder showed extensive lesions of mastitis. This case was treated during an extremely cold spell of weather, which may have contributed to the pneumonia.

In May 1940, while the heifers in this herd were being examined for pregnancy, a heifer was found with a swollen left rear quarter.

The swelling was hard, about the size of a baseball and moderately painful. The exudate from this quarter gave a pure culture of slow-growing, minute, pin-point colonies of an alpha hemolytic streptococcus. Attempts to develop a bacteriophage for this organism were unsuccessful. The quarter was treated by stripping. The swelling gradually subsided, but there appears to be a considerable amount of proliferated connective tissue within the sinus. This animal will be kept under observation in order to determine the status of her udder at calving.

Etiology

S. aureus was the predominating microörganism isolated from the udders of the heifers with mastitis on the three farms. In the majority of the animals this organism was isolated in pure culture. Streptococci were isolated from the udder exudate of ten of the 24 heifers studied in this series of cases. In four of these heifers streptococci were the only organisms isolated. In five heifers streptococci were isolated in pure culture from certain infected quarters, whereas other quarters gave pure cultures of hemolytic *S. aureus*. In one heifer the exudate gave a mixture of streptococci and staphylococci.

The habitat of *S. aureus* is the skin and mucous membranes. In the lactating cow the interlobular ducts and lactiferous sinus may be regarded as the normal habitat as well. Ward[4] concluded from his studies that "milk when secreted by the glands of the healthy cow udder is sterile. It may, however, immediately become contaminated by the bacteria which are normally present in the smaller milk ducts of the udder." Conn *et al*[5] observed that cultures classed as micrococci possess characteristics agreeing essentially with those of the pathogen "Staphylococcus pyogenes aureus." Evans,[6] in a study of the bacteria of milk freshly drawn from normal udders, reported that micrococci were found in 58.8 per cent of the samples varying from few to 80,000 per cubic centimeter. Of 185 cultures, 95 were regarded as *S. aureus*. While the majority were nonvirulent, about 10 per cent of the cultures of micrococci were hemolytic on cow's blood agar, produced pigment, fermented mannitol and were virulent for rab-

[4]Ward, A. R.: The invasion of the udder by bacteria. Cornell Univ. Agr. Exp. Sta. Bul. 178 (1900).

[5]Conn, H. W., Esten, W. M., and Stocking, W. A.: A classification of dairy bacteria. Connecticut Agr. Exp. Sta., 18th Ann. Rpt. (1906).

[6]Evans, A. C.: The bacteria of milk freshly drawn from normal udders. J. Inf. Dis., xviii (1916), pp. 437-476.

bits. The hemolytic *S. aureus* strains appeared to be more pathogenic than the nonhemolytic *S. albus* strains. As most of the udder micrococci studied appeared to be of the same type as those commonly found on the skin and mucous membranes of man and animals, Miss Evans suggested dropping the several specific terms applied to micrococci of the udder. Steck[7] found that certain udder quarters remained sterile for long periods; others continued to harbor relatively large numbers of bacteria, including *S. aureus* and *S. albus*. He considered such organisms capable of setting up a mild inflammatory reaction in the gland.

Several investigators have reported *S. aureus* as the cause of mastitis. Plastridge *et al*[8] stated that staphylococci of the bovine udder are of interest to the dairy industry for several reasons: (1) They are important causative agents in mastitis, second only to streptococci in this respect; (2) they may lower the quality of the herd milk by causing high leucocyte and high bacterial counts; and (3) "Available evidence suggests that under certain conditions some strains of udder staphylococci may be capable of producing substances toxic to man." Little and Foley[9] state, "Mastitis associated with staphylococci, although less common than streptococcic mastitis, may occasionally become very acute," and "The affected animal may completely recover, or a partial or serious injury to the diseased quarter may remain, or death may occur."

Guillebeau[10] found that staphylococci were responsible for severe udder inflammations and that some types liquefied gelatin. Jones[11] considers that next to streptococci the micrococci were most frequently involved in mastitis, producing, as a rule, mild inflammation in which the secretion contained flakes for a day or two, followed by recovery and frequently by the disappearance of the organisms from the secretion. In a few instances micrococci were associated with acute mastitis which became chronic. The cultures possessed the usual characteristics of *S. aureus* and *S. albus*. Minett[12] reported that "cases of mastitis, acute and chronic, caused by pathogenic staphylococci (*S. aureus*) have been found to be of such a nature as to be confused with mastitis caused by streptococci. . . . In its commonest form the disease is chronic with stroma being ultimately involved. Enduring infection leads to diffuse induration of the gland with some decrease in milk yield and qualitative changes in the milk and excessive alkalinity, increase in leukocytes, a lowered specific gravity, and solids-not-fat content." Hopkirk[13] observed several herds in New Zealand in which staphylococcic mastitis was as common as the streptococcic form.

It is not difficult to reproduce the disease in healthy animals with pure cultures isolated from infected animals. Little and Foley[9] have shown that staphylococci associated with abscesses of the skin of the udder, when introduced into the udder, are capable of producing an acute severe infection presenting the same clinical manifestations of disease as occur in field cases. Carpenter[14] inoculated 2 cc. of 24-hour broth culture of nonhemolytic *S. aureus*, obtained from a case of mastitis, into the udders of three heifers and two cows. Four animals reacted severely, showing a high temperature with loss of appetite for 36 hours following the inoculation. Ten days later, one cow died from septicemia resulting from the infection of the udder and at autopsy staphylococci were isolated from

[7]Steck, W.: Schweiz. Arch. f. Tierh., lxii (1920), p. 525. Cited by Plastridge *et al*.[8]

[8]Plastridge, W. N., Weirether, F. J., and Williams, L. F.: Observations on staphylococci associated with bovine mastitis. J. Bact., xxxi (1936), pp. 88-89.

[9]Little, R. B., and Foley, E. J.: Staphylococci associated with mastitis. J.A.V.M.A, lxxxvii (1935), p. 537.

[10]Guillebeau, A.: Studien über die Milchfehler und Euterentzundungen bei Rindern und Ziegen. Landw. Jahrb. Schweiz., iv (1890), p. 27. Cited by Little and Foley.[9]

[11]Jones, F. S., and Little, R. B.: The bactericidal property of cow's milk. J. Exp. Med., xiv (1927), p. 313.

[12]Minett, F. C.: Studies on bovine mastitis. XII. Mastitis due to staphylococci. J. Comp. Path. & Therap., l (1937), p. 181.

[13]Hopkirk, C. S. M.: Research report. New Zealand Dept. Agr. (1935). Cited by Plastridge *et al*.[8]

[14]Carpenter, C. M.: Experimental production of bovine mastitis with streptococci and other bacteria. J. Inf. Dis., xxxi (1922), p. 1.

all the tissues of the body. In three animals abscesses appeared in the udders.

PROPERTIES OF THE STAPHYLOCOCCI ISOLATED

Bacteriologic study of the milk of normal cows has revealed that staphylococci constitute the major part of the normal udder flora and that such microörganisms were nonpathogenic. Later work revealed that some strains of staphylococci were capable of inducing a mild or severe mastitis. It, therefore, became a problem in fundamental research to determine the properties which distinguish the pathogenic from the nonpathogenic strains. Such research is fundamental to the diagnosis and control of staphylococcic mastitis.

Since the work of Dudgeon[15] (1908) and others, many workers regard all staphylococci as members of one species. The pathogenic strains possess properties which distinguish them more or less from the nonpathogenic strains. In passing from pathogenic types to the nonpathogenic types, every degree of variation occurs with a progressive weakening of pathogenicity and biochemic activity until reaching the saprophytic type commonly found in the skin, in the udder of cows, and in dust.

The properties which are of greatest diagnostic value in differentiating the pathogenic types from the nonpathogenic types are (1) hemolysin production, (2) blood-plasma coagulation, (3) gelatin liquefaction, (4) violet or orange color on crystal-violet agar, (5) mannitol fermentation, (6) changes of litmus milk, and (7) rabbit-skin reactions.

Hemolysin Production.—S. aureus produces two distinct toxins—alpha and beta. Minett[16] found that beta toxin production is a characteristic feature of hemolytic staphylococci of animal origin. Most of the cultures obtained from bovine mastitis produce alpha and beta toxin or beta toxin, and a few produce alpha toxin only. Because ox blood is susceptible to both alpha and beta hemolysis, Minett recommends it for use in blood-agar plates for the isolation of pathogenic staphylococci from milk. Plastridge *et al*[8] observed a fairly close correlation between the ability of cultures to hemolyze cow's blood and high leucocyte counts in milk. In a later report Plastridge *et al*[17] concluded that the nonhemolytic strains produce a mild irritation of the udder, whereas the hemolytic strains produce irritation sufficient to cause a marked increase in leucocyte count and chloride content of the udder secretion. Hopkirk[13] reported that the nonhemolytic staphylococci were occasionally found to be associated with a mild mastitis characterized by an increased output of leucocytes. Of 20 cultures from bovine mastitis studied by Bryce and Rountree,[18] three produced alpha hemolysin, seven beta hemolysin, five alpha and beta, and five were nonhemolytic. They also observed that ox blood was hemolyzed by both alpha and beta hemolysins. Julienelle[19] found that in general the least hemolytic of cultures he studied were the least virulent; however, some strains giving rapid hemolysis were saprophytic.

Chapman *et al*[20] have concluded that "a knowledge of the hemolytic activity of staphylococci is insufficient as a single serologic test of pathogenicity of this species." As shown in table I, twelve of the 14 animals with acute mastitis in our study gave hemolytic staphylococci; eleven gave beta hemolysin and one alpha hemolysin; two of the animals gave nonhemolytic staphylococci.

Not all of the staphylococci isolated from this group of mastitis cases which gave hemolysis on ox-blood plates were capable of hemolyzing ox blood in tryptose broth. Plastridge *et al*[8] made similar ob-

[15]Dudgeon, L. S.: The differentiation of the staphylococci. J. Path. & Bact., xii (1908), pp. 242-257. Cited by Plastridge *et al*.[8]

[16]Minett, F. C.: Staphylococci from animals with particular reference to toxin production. J. Path. & Bact., xlii (1936), p. 247.

[17]Plastridge, W. N., Anderson, E. O., Williams, L. F., and Wierether, F. J.: Infectious bovine mastitis. 7. Characteristics of udder staphylococci. Connecticut (Storrs) Agr. Exp. Sta. Bul. 231 (1939).

[18]Bryce, L. M., and Rountree, P. M.: The production of B-toxin by staphylococci. J. Path. & Bact., xliii (1936), pp. 173-189.

[19]Julienelle, L. A.: Studies on hemolytic staphylococci. Hemolytic activity—biochemical reactions—serologic reactions. J. Inf. Dis., xxxi (1922), pp. 256-284. Cited by Plastridge *et al*.[17]

[20]Chapman, G. H., Berens, C., Peters, A. and Curcio, L.: Coagulase and hemolysin tests as measures of the pathogenicity of staphylococci. J. Bact., xxviii (1934), pp. 343-363.

servations on their study of 465 cultures. Of these, 145 produced weakly hemolytic colonies on blood agar and hemolyzed blood broth; 67 produced weakly hemolytic colonies but failed to hemolyze blood broth.

Blood-Plasma Coagulation. — Some strains of staphylococci produce a plasma-clotting substance known as coagulase, which is probably independent of hemolysin. Cruickshank[21] concluded that because coagulase was found to be present more constantly in actively pathogenic strains of staphylococci than hemolysin, the coagulase test should be used as a routine test for pathogenicity. Chapman *et al*[20] concluded: "A determination of coagulase activity is an important adjunct to the hemolysis test. Coagulating strains are usually pathogenic regardless of their hemolytic activity." Plastridge *et al*[17] state that their "results show that the coagulase test, when made with human plasma, is effective in separating cultures of udder staphylococci which possess little or no pathogenicity from those which possess a relatively high degree of irritating ability for the bovine udder. The test was equally efficient when applied to nonhemolytic, weakly hemolytic and hemolytic cultures. . . . The ability of recently isolated udder staphylococci to coagulate human plasma was found to be more closely related to irritating ability than any other property. They found that bovine plasma is decidedly less sensitive to the coagulase of udder staphylococci than is human plasma and that rabbit's blood plasma may be used in place of human plasma. Our studies have confirmed this observation and we have discontinued the use of ox-blood plasma, substituting in its place rabbit-blood plasma. Ten of the 14 strains reported in this series produced "coagulase" for rabbit's blood.

Gelatin Liquefaction.—Staphylococci which liquefy gelatin show a tendency to be more irritating to the bovine udder than nonliquefying strains, according to Plastridge *et al*,[17] but the relation of the gelatin-liquefying properties of udder staphylococci to evidence of mastitis was not sufficiently close to justify the use of the gelatin liquefaction test alone as evidence of pathogenicity. In this study approximately 50 per cent of the cultures were able to liquefy gelatin.

Violet or Orange Color on Crystal-Violet Agar.—Chapman and Berens[22] reported that when crystal violet was added to solid media, it was noted that strains reacting positively to hemolysis and coagulase tests produced violet growths, or golden growths tinged with violet, while those strains which reacted negatively to hemolysis and coagulase tests produced white or pale growths. Plastridge *et al*[17] were somewhat disappointed with this test because the proportion of coagulase-negative cultures which gave violet-colored growth was larger than expected—77.3 per cent—and the proportion of coagulating cultures recorded as positive was smaller than expected — 85.5 per cent. We were likewise disappointed with this test, as only 50 per cent of our cultures which were positive on rabbit plasma gave violet-colored growth on crystal-violet media.

Mannitol Fermentation.—Plastridge *et al*,[17] in their studies on pathogenic and nonpathogenic staphylococci, concluded that inability to ferment either lactose or mannitol alone usually indicates relatively low virulence, but that ability to act upon the two carbohydrates is not limited to cultures of relatively high virulence. Eight of the 14 cultures obtained from our mastitis cases fermented mannitol.

Changes of Litmus Milk.—Plastridge *et al*[17] concluded that for practical purposes their results show that udder staphylococci which fail to alter litmus milk or which cause peptonization only usually possess slight, if any, irritating ability for the bovine udder. All of our cultures were capable of changing litmus milk. However, as pointed out by Plastridge, the differential value of reactions produced in litmus milk by udder staphylococci is limited, be-

[21]Cruickshank, R.: Staphylocoagulase. J. Path. & Bact., xlv (1937), p. 295. Cited by Plastridge *et al*.

[22]Chapman, G. H., and Berens, C.: Crystal violet agar as a differential medium for staphylococci. J. Bact., xxix (1935), p. 437.

cause a large percentage of nonpathogenic strains also acidify litmus milk.

Rabbit-Skin Tests.—Preliminary studies indicate that intracutaneous injection into rabbits of cultures of *S. aureus* may be of value in the differentiation of pathogenic and nonpathogenic strains of the organism. Some pathogenic strains produce a much larger skin lesion than others. This is due to the so-called spreading factor produced by the organism, which has been described by Duran-Reynals.[23] The results of this test are recorded in table I as negative, 1+, 2+, 3+, and 4+, which are interpreted as follows: A negative reaction is one in which there apears a slight amount of erythema at the point of injection with no abnormal swelling, except that formed by the formation of the bleb which disappears in two or three days. A 1+ reaction is one in which there appears an increase in the amount of erythema with abscess formation and swelling over an area not exceeding 1 to 1½ cm., and which persists for seven to ten days. A 2+ reaction is one in which the swelling and erythema does not exceed that of a 1+ reaction, but in which slight necrosis is observed at the point of injection which persists for ten to 14 days. A 3+ reaction is one in which the swelling and erythema extends over an area 1½ cm. by 3 cm. The area of necrosis may involve one third of the area of the abscess and the reaction may persist for two or three weeks. A 4+ reaction is one in which the area of swelling and erythema extends over an area 4 cm. by 6 cm. or over. The area of necrosis involves two thirds of the area of the swelling, and the reaction persists for three or four weeks. All of the cultures in this study except one gave positive reactions when injected into the skin of rabbits. As this study was not made until some of the cultures had been held for some time in the laboratory, these may have lost some of their pathogenicity.

[23]Duran-Reynals, F.: Studies on a certain spreading factor existing in bacteria and its significance for bacterial invasiveness. J. Exp. Med., lviii (1933), pp. 161-181. Cited by Bronfenbrenner and Sulkin.[29]

AGGLUTINATION REACTIONS

All of the animals reported in this study, which were shown to be infected with *S. aureus*, developed positive agglutination reactions with *S. aureus* antigen. As some of the animals were treated with bacteriophage prior to testing the blood serum for the presence of *S. aureus* agglutinins, we have eliminated from this consideration animals so treated because of the possibility that the bacteriophage may influence the agglutination titer. For purposes of comparison the blood sera of noninfected heifers and young bulls were tested for *S. aureus* agglutinins. The results of this study are shown in table III.

In some of the infected animals the specific agglutinins did not appear in the blood stream until some little time after the onset of the mastitis. This test, therefore, is not always an aid in early diagnosis of staphylococcic mastitis. Furthermore, since staphylococcic infections involving other parts of the body, as an infected foot, etc., give rise to the production of staphylococcic agglutinins, a positive reaction may not indicate necessarily an udder infection.

BACTERIOPHAGE ACTION

All of the strains of staphylococci isolated in this group of clinical cases were lysable by our polyvalent *S. aureus* bacteriophage. This property gives promise of possessing diagnostic value in differentiating pathogenic and nonpathogenic strains. In a recent survey the milk of individual quarters from 150 cows examined on ox-blood plates gave 114 *S. aureus* cultures. All of these cultures were subjected to the action of polyvalent staphylococcus bacteriophage for their lysogenic properties. Eighty-two of the cultures were lysable and 32 were nonlysable. Preliminary skin tests in rabbits appear to indicate that the lysogenic cultures are more pathogenic than the nonlysogenic cultures. The lysable strains produced an extensive area of inflammation with marked necrosis as compared with the nonlysable strains which produced local erythema with only slight or no necrosis. These preliminary studies also indicate no definite correlation

TABLE III—Agglutination Reactions

Animal	Blood-Serum Dilutions					
	1:50	1:100	1:200	1:400	1:800	1:1,600
Infected Animals						
329	4+	4+	4+	4+	2+	1+
417	4+	4+	3+	2+	2+	—
421	4+	4+	3+	2+	—	—
433	4+	4+	4+	3+	2+	—
477	4+	4+	2+	—	—	—
Noninfected Animals						
Bull 1	2+	1+	—	—	—	—
Bull 2	2+	1+	—	—	—	—
Bull 3	1+	—	—	—	—	—
Bull 4	3+	—	—	—	—	—
Bull 5	1+	—	—	—	—	—
Heifer 1	1+	—	—	—	—	—
Heifer 2	2+	1+	—	—	—	—
Heifer 3	1+	—	—	—	—	—
Heifer 4	±	—	—	—	—	—
Heifer 5	—	—	—	—	—	—

between the hemolytic and lysogenic properties of the udder staphylococci. Of the 82 lysable cultures, 18, or 21.9 per cent, were nonhemolytic, whereas of the 32 nonlysable cultures, 15, or 46.8 per cent, were hemolytic. It is planned to report this study when the work is finished.

Streptococci Isolated

The streptococci isolated from this group of clinical cases of mastitis in heifers are shown in table IV. Typical mastitis streptococci (*Streptococcus agalactiae*) were isolated in only one heifer with clinical mastitis. This heifer (No. 480) was observed on June 16, 1939, to have a swollen udder, involving the left side. The left fore quarter contained a thick, pus-like exudate which gave a pure culture of a beta hemolytic streptococcus, producing minute, pinpoint, slow-growing colonies (table IV, W28d1). The left rear quarter contained a more fluid exudate which gave a pure culture of *S. aureus*. Bacteriophage, specific for both organisms, was produced and treatment with the mixed bacteriophage was started on June 23. Before the bacteriophage was injected, samples of the exudate were collected. Both involved quarters gave pure cultures of *S. aureus*. She received daily injections of the mixed phage for seven days. The swelling and amount of exudate rapidly subsided in both quarters, the exudate became sterile and on the seventh day her recovery was considered complete. On August 7, 1939, the left fore quarter was again observed to be swollen. The exudate gave a pure culture of an alpha prime hemolytic streptococcus, which upon the Lancefield precipitin test and the rapid tube agglutination test proved to be group B (table IV, W28d2).

Lancefield group C streptococci were found in two of the heifers. In one heifer (No. 426) this was the only organism isolated. In the second heifer (No. 478) this organism was isolated from the right rear quarter. The swelling in this quarter was hard, hot and painful. The remaining quarters were less swollen, soft and gave a watery, straw-colored exudate. These quarters gave pure cultures of *S. aureus*.

From the udder exudate in seven heifers with clinical mastitis an unusual streptococcus was isolated. In three of the animals this was the only organism isolated. In three others the organism was isolated in pure culture from certain quarters, whereas *S. aureus* was found in other quarters. In one animal it was mixed with *S. aureus*. This unusual streptococcus appears to be an organism not previously described, although it resembles in some respects the minute streptococci of human origin reported by Long and Bliss.[24] One strain W41 obtained in pure culture from animal 479 (see pages 20-21), which is typical for the group, reproduced mastitis

TABLE IV—Streptococci Isolated from Heifers

Animal or Culture	Isolation	Morphology	Colonies on Ox-Blood Tryptose Agar	Litmus Milk			Sodium Hippurate	Mannitol	Inulin	Salicin	Trehalose	Sorbitol	Aesculin	Addition of Sterile Blood Serum	Serologic Group Lancefield
				Acid	Reduction	Curd									
L-4	Pure culture in RR; mixed with *Staph. aureus* in RF	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	—	—	+	—	—	—	+	—	—	Stimulated rapid growth	Unclassified; not A, B or C
482	Pure culture in LR	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	—	—	+	—	—	—	—	—	—	Stimulated rapid growth	Unclassified; not A, B or C
479	Pure culture in RF	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	+	+	+	—	—	—	+	—	—	Stimulated rapid growth	Unclassified; not A, B or C
480 W28d1	Pure culture in LF; *Staph. aureus* in LR	Short chains small cocci	*β* hemolysis; minute pin-point, slow-growing	A	—	+	+	+	—	—	+	—	—	Stimulated rapid growth	Unclassified; not A, B or C
B-3	Pure culture in LR	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	+	+	—	—	—	—	—	—	—	Stimulated rapid growth	Unclassified; not A, B or C
446	Pure culture in RR and LF; *Staph. aureus* in LR	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	+	+	+	+	—	—	+	—	—	Stimulated rapid growth	Unclassified; not A, B or C
B-2	Mixed with *Staph. aureus*	Short chains small cocci	*a* hemolysis; minute pin-point, slow-growing	A	—	—	+	+	+	+	+	+	+	Stimulated rapid growth	Unclassified; not A, B or C
480 W28d2	Pure culture in LF	Long chains in pairs	*a* hemolysis; rapid growth	A	—	—	+	+	—	—	+	—	—	Rapid growth without serum	B
426	Pure culture in LR	Short chains small cocci	*a* hemolysis; slow-growing	A	—	—	—	—	—	—	+	—	—	Stimulated rapid growth	C
478	Pure culture in RR; others *Staph. aureus*	Long chains in pairs	*a* hemolysis; minute slow-growing	A	—	—	+	—	—	—	+	—	—	Stimulated rapid growth	C

when injected into the udder of a normal heifer.

Considered as a group, the growth on blood-agar plates (tryptose agar plus 10 per cent citrated ox blood) of these organisms is characterized by the minute character of the colonies. The diameter of surface colonies does not exceed 1 mm. The rate of growth in primary isolations is very slow, when the milk or udder exudate from the infected animals is streaked on the blood plates. There is no perceptible growth within the first 24 hours of incubation at 37° C. However, after 48 hours of incubation, the colonies appear as small, pin-point growths with little or no change of the blood. Upon further incubation some of the strains produce a small zone of hemolysis, others produce a small zone of incomplete hemolysis, and others produce a slight discoloration of the blood, accompanied by a small superficial zone of hemolysis.

The shape of the surface colonies is circular and convex. When viewed with the low-power magnification, the edge is entire, the surface is glistening and the internal structure is finely granular. The morphology of the cells was observed from Gram's stain preparations of 48-hour growth on blood-agar plates. The cells are small and spherical or ovoid, occurring in pairs or short chains of not more than eight to ten cells. When the cells are elongated, the axis of the cells is usually in the axis of the chain. They are Gram positive.

The fermentation reactions in general

[20]Long, P. H., and Bliss, E. A.: Studies on minute hemolytic streptococci. J. Exp. Med., lx (1934), p. 619.

were not in agreement. Mannitol was fermented by three of the seven cultures of the unusual streptococcus. All of the cultures except one were negative in inulin, salicin, sorbitol and aesculin. Trehalose was fermented by five of the cultures and negative in two. Sodium hippurate was hydrolyzed by six of the seven cultures. The reaction in litmus milk was characterized by some variability in the ability to reduce litmus and to coagulate the milk; however, all cultures produced acid in this medium, which indicates their ability to ferment lactose.

The antigenic constitution of all streptococci isolated from the heifers was studied by means of the Lancefield[25] precipitin test and the rapid tube agglutination test as reported by Stableforth.[26] One of the cultures was established as group B, and two cultures were group C. None of the seven remaining cultures reacted with any of the sera of groups A, B and C. An antiserum of high titer against culture W41 obtained in pure culture from heifer 479 and which reproduced the disease in a normal heifer was prepared according to the method of Lancefield. Four of the cultures of the unusual streptococcus found in the heifers gave a positive reaction to this serum. In three of these animals (Nos. 482, 479 and B3) this was the only organism isolated. In one heifer (No. 480) it was isolated in pure culture from one infected quarter. Three of the heifers were members of the herd of farm I and one was a member of the herd of farm III. Studies are now under way in an endeavor to establish the Lancefield group of these organisms.

Pathology

Fibrosis was the most pronounced pathologic change observed in this series of cases. In some of the heifers the lactiferous sinuses and ducts were completely obliterated by proliferated connective tissue. In some animals the ducts became completely closed, leading to the condition which dairymen term "blind" quarters. On gross examination the alveoli appeared to have undergone atrophy and there was excessive proliferation of connective tissue throughout the interlobular spaces. The fibrosis could be distinctly palpated in the living animal. In animal 441, which developed a swollen left fore quarter about three weeks before calving, it was impossible to remove the milk from this quarter after calving because of the extensive fibrosis. The right fore quarter was similarly involved and the teat duct was completely occluded. Microscopic examination of these quarters revealed some portions of the gland were microscopically normal. Other portions showed inflammatory reactions characterized by interlobular infiltration of polymorphonuclear leucocytes and recent proliferation of interlobular connective tissue cells.

Two of the heifers developed gangrenous quarters. In animal 446 both right quarters were almost entirely necrotic at the time of slaughter. Microscopic examination revealed only the outlines of the glands, with only blood vessels, connective tissue strands and fat tissue remaining. Details of cellular structure were lost. Numerous bacterial colonies were present within the acini. A few viable connective tissue cells had undergone proliferation around some of the blood vessels. Numerous polymorphonuclear and large mononuclear leucocytes were diffusely infiltrating the tissue.

Treatment

Since mastitis is not a specific infectious disease caused by a single variety of microörganism, but may be caused by a wide variety of microörganisms, it is important in discussing the treatment of this disease to indicate the specific bacterial type of mastitis which is being treated. The bacteriophage employed in treating this series of cases was specific, in every instance, for the type of infection found in the animal.

In staphylococcic mastitis natural recovery in mature cows has been observed by several workers. Jones[27] has reported the

[25]Lancefield, R. C.: A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med., lvii (1933), pp. 571-595.

[26]Stableforth, A. W.: Studies on bovine mastitis. VII. The serological characteristics of mastitis streptococci. J. Comp. Path. & Therap., xlv (1932), pp. 194-211.

[27]Jones, F. S.: Studies in bovine mastitis. III. Infection of the udder with micrococci and other microörganisms. J. Exp. Med., xxviii (1918), pp. 721-734. Cited by Plastridge *et al.*[8]

presence of an inhibitory substance in the milk against udder staphylococci. We have observed natural recovery in mature cows infected with staphylococcic mastitis, and in the milk of these animals we were able to demonstrate an inhibitory (lysogenic) substance which was unquestionably a bacteriophage. In the heifers, which were infected with *S. aureus*, the bacteriophage specific for the staphylococcus carried in their udders could not be demonstrated except in one heifer which was milking at the time of becoming infected.

Clinical experience with udder infections in heifers indicates that unless the infection is destroyed early in the disease, the udder will be seriously damaged by fibrosis and possibly necrosis. As treatment with warm fomentations and frequent stripping of the infected quarters was not successful in preventing the proliferation of connective tissue and since natural bacteriophage was demonstrated in the milk of mature cows infected with *S. aureus*, it was decided to try treating the infected heifers with bacteriophage specific for the type of infection found in each infected heifer. We know of no previous work where bacteriophage therapy has been employed in infections of the udder. Bronfenbrenner and Sulkin[28] failed to detect any therapeutic effect of the bacteriophage in the treatment of staphylococcic skin infection in experimentally infected animals.

Daily injections of a bacteriophage, giving a 4+ lysis against the specific staphylococcus or streptococcus carried by the animal, into the lactiferous sinus were followed by rapid recovery in the infected heifers with nonfunctioning udders in all but one animal. In heifers with functioning udders the bacteriophage therapy was of doubtful value. The bacteriophage, in most instances, was well tolerated by the animals. It was injected under aseptic precautions directly into the lactiferous sinus *via* the teat duct in doses of 2.5 cc. to 10 cc. in heifers with nonfunctioning udders and in much larger doses in heifers which were secreting milk. In the majority of animals no reactions were observed following the introduction of the bacteriophage in the udder. A few heifers developed edematous swelling of the udder shortly after receiving the bacteriophage. These swellings subsided within 24 hours. In two heifers, which developed swelling of the udder following the injection of bacteriophage, the swelling did not subside rapidly. Heifer 479, in which the exudate gave a pure culture of an alpha hemolytic streptococcus producing minute, pin-point colonies, developed a pronounced swelling of the udder which terminated in multiple abscess formation. Heifer 446, which developed mastitis at calving time due to a mixed infection consisting of *S. aureus* and a streptococcus producing minute, pin-point alpha hemolytic colonies, developed a pronounced swelling of the udder which terminated in necrosis. These unfavorable reactions may have been caused by the "spreading factor" which has been described by Bronfenbrenner and Sulkin.[29]

Two methods of preventive treatment have been followed.

1) Segregation of infected animals: In herds where staphylococcic or streptococcic mastitis is a problem, an attempt may be made to control the spread of the infection by frequent bacteriologic examinations of the milk of all cows and the udder secretions of all heifers showing abnormal swellings of the udder upon ox-blood agar. As many cows carry in their udders staphylococci which are nonpathogenic, research should be continued in an effort to devise tests whereby the carriers of pathogenic strains of staphylococci may be differentiated from those carrying harmless strains of the microörganism. The ox-blood-agar plates, rabbit-blood-plasma coagulation, the presence of alpha hematoxin, and the rabbit-skin test offer the best means available for detecting carriers of pathogenic staphylococci. For the pathogenic strep-

[28]Bronfenbrenner, J., and Sulkin, S. E.: Bacteriophage therapy. I. Effect of bacteriophage on cutaneous staphylococcus lesions in rabbits. J. Inf. Dis., lxv (1939), p. 53.

[29]Bronfenbrenner, J., and Sulkin, S. E.: Bacteriophage therapy. III. On the nature of the deleterious effect of the local application of staphylococcus bacteriophage. J. Inf. Dis., lxv (1939), p. 64.

tococci primary isolations should be made upon ox-blood agar followed by a study of the fermentation reactions and their antigenic constitution. In three of our experimental herds we are endeavoring to control mastitis by frequent ox-blood-agar plating and the other tests and segregation of the infected animals. Plastridge *et al*[8] have concluded from their studies on pathogenic udder staphylococci that "in the routine examination of milk, udder staphylococci which produce hemolytic colonies on cow's blood or which are associated with leucocyte counts of 50,000 or more per cc., may be considered as probably pathogenic, provided that high leucocyte counts resulting from early or late lactation are excluded."

2) External application of bacteriophage: On one farm in a lot of 31 yearling heifers in which several cases of mastitis appeared, an attempt was made to prevent the spread of the infection by spraying the udders with bacteriophage. The heifers were on pasture and as flies were observed to be present at times on the teats, it was suspected that these vectors may be responsible for the spread of the infection. Several workers have incriminated flies in the spread of mastitis. In work not yet reported, Dr. Harry E. Ewing, Jr., of this department, tested 152 flies trapped in a dairy barn which housed 61 milking cows, 34 of which were shedding mastitis streptococci (*Str. agalactiae*), five were shedding pathogenic *S. aureus*, one was shedding both streptococci and staphylococci, and 21 were mastitis negative. No mastitis streptococci were found on any of the flies, but two flies carried pathogenic *S. aureus*. Nonpathogenic strains of *S. aureus* were found on many of the flies. Sanders[30] recently succeeded in transmitting mastitis from infected to experimental noninfected cows by the common housefly, *Musca domestica*. He concluded that the "results of these tests show conclusively that *M. domestica* is a natural vector of bovine mastitis."

[30] Sanders, D. A.: *Musca domestica* a vector of bovine mastitis (preliminary report). J.A.V.M.A., xcvi (1940), pp. 120-122.

A large supply of polyvalent bacteriophage giving a 4+ lysis for all strains of microörganisms isolated from these heifers was produced and applied twice weekly by means of an atomizer. The value of this treatment can not be determined accurately as no heifers were left untreated as controls. In the group of 31 heifers, three animals developed mastitis before the external treatments were started; three clinical cases developed subsequently; two had damaged udders ("blind" quarters) when fresh, indicating early infection although they showed no clinical mastitis; and 23 have freshened with normal udders.

Summary and Conclusions

1) Mastitis was studied in heifers in three dairy herds. In one herd the disease assumed epizoötic proportions, whereas in the other two herds only sporadic cases occurred.

2) In herd I, mastitis appeared suddenly, during the summer of 1938, in the mature cows of the herd and later in the heifers.

3) Twenty heifers were known to have been infected in herd I, four in herd II, and two in herd III.

4) *Staphylococcus aureus* was the predominating causative agent.

5) Streptococci were isolated from the udder exudate of infected quarters in ten heifers.

6) In four heifers streptococci were the only organisms isolated; in five heifers streptococci were isolated in pure culture from certain infected quarters, whereas other infected quarters gave pure cultures of *S. aureus;* and in one heifer the streptococci were mixed with *S. aureus*.

7) Of the ten heifers infected with streptococci only one gave typical mastitis streptococci (Lancefield group B), two heifers gave Lancefield group C streptococci, and seven heifers gave an unusual streptococcus.

8) The unusual streptococcus appears to be an organism not previously described in connection with mastitis.

9) Of the seven heifers giving the new streptococcus, this was the only organism isolated in three heifers; in three others it was isolated in pure culture from certain quarters and *S. aureus* in other quarters; and in one heifer it was mixed with *S. aureus*.

10) The new streptococcus was capable of reproducing mastitis in an experimental heifer.

11) Culturing the milk or exudate upon ox-blood-tryptose-agar plates was found to be the most satisfactory method for the isolation of pathogenic streptococci and staphylococci.

12) Abnormal swelling of the udder of heifers may be physiologic or pathologic; if physiologic, the exudate is usually more viscid, may clot upon standing, and upon bacteriologic examination will be sterile.

13) A few of the infected heifers gave no indications of being infected until calving time, when they freshened with one or more "blind" quarters.

14) Udder infections in heifers with pathogenic staphylococci or streptococci, unless destroyed by natural recovery or by treatment, produce serious pathologic changes in the udder.

15) Fibrosis was the most common pathologic change in this group of animals; necrosis of the infected quarters occurred in two heifers, and abscess formation occurred in one heifer.

16) When the fibrosis is well established the lactiferous sinus and sometimes the teat duct become obliterated by proliferated connective tissue producing what dairymen term "blind" quarters.

17) Natural recovery was observed in mature cows infected with staphylococcic mastitis.

18) Generalized infection following mastitis, in which *S. aureus* was isolated in pure culture from the blood stream, was observed in one heifer.

19) In natural recovery in mature cows with staphylococcic mastitis bacteriophage specific for the invading organism was demonstrated in the milk.

20) Treatment consisting of frequent stripping of the infected quarters and the application of warm fomentations was unsuccessful in promoting recovery in heifers with nonmilking udders.

21) Bacteriophage therapy gave satisfactory results in infected heifers with nonfunctioning udders, but was of doubtful value in infected heifers with functioning udders.

An Outbreak of Bovine Pseudorabies, or "Mad Itch"*

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A FILTRABLE VIRUS indistinguishable from the virus of pseudorabies has been isolated for the first time in Illinois from the spinal cord of a clinically affected steer.[1] This disease as previously reported in cattle in Iowa by Shope[2] also is designated as "mad itch" and infectious bulbar paralysis. The causative virus, according to Shope,[3] is indistinguishable from that of Aujeszky's disease, which has been recognized for many years in Europe. For a comprehensive review of the literature on this disease, the reader is referred to a relatively recent article by Galloway.[4]

It is the purpose of this report to record certain field and laboratory observations made during a spontaneous outbreak of bovine pseudorabies with the view of assisting the clinician in prompt recognition and suppression of the disease. Attention is invited to the advantages of selecting a typically affected live animal for laboratory diagnostic studies. The disease was not identified in Illinois until a live affected animal was supplied for study. However, a field autopsy might be performed and the spinal cord aseptically removed and brought to the laboratory for diagnosis.

The identification of the virus of pseudorabies in cattle in Illinois conjectures the part played by this virus in analogous undiagnosed syndromes in cattle which have been reported occasionally by Illinois veterinarians for more than a quarter of a century. It is reasonable to believe that outbreaks of pseudorabies have been indefinitely or erroneously diagnosed in Illinois, as indicated by Murray[5] in Iowa. Furthermore, the epizoölogical features of the outbreak coming to our attention support the belief in the active rôle of the porcine host as a carrier of the virus.[6] At the time of and preceding the development of the outbreak described herein, approximately 75 apparently healthy hogs were in the feedlot with the cattle. All of the affected steers had been continuously fed in two lots with these animals. Following the removal of the hogs from the feedlot, only one clinical case of the disease developed in cattle. For the remainder of the feeding period, the hogs were separated from the cattle but were allowed access to the lots vacated by the cattle in alternate weeks.

It is also recognized that the rat may play a part in the epizoölogy of pseudorabies and on premises where the disease occurs, this intermediate host should not be disregarded.[7] In fact, the alertness of the clinician in recognizing the clinical features of pseudorabies in cattle and the potential rôle of apparently healthy swine or of rats in perpetuating the disease may greatly influence the extent of the losses sustained.

HISTORY AND SYMPTOMS

On February 12, 1940, a live Hereford steer, in good flesh and weighing about 1,000 lb., was delivered to the Laboratory of Animal Pathology and Hygiene. Symp-

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†Assigned to the Laboratory of Animal Pathology and Hygiene, University of Illinois, by the Illinois State Department of Agriculture to assist in diagnostic work.

[1]Graham, R., Morrill, C. C., and Boley, L. E.: Bovine pseudorabies or "mad itch" virus. Sci., xci (1940), pp. 623-624.

[2]Shope, R. E.: "Mad itch" of cattle. Sci., lxxii (1930), p. 559.

[3]Shope, R. E.: An experimental study of "mad itch" with special reference to its relationship to pseudorabies. J. Exp. Med., liv (1931), pp. 233-248.

[4]Galloway, I. A.: Aujeszky's disease. Vet. Rec., I (1938), pp. 745-762.

[5]Murray, C.: Mad itch. Cornell Vet., xxiii (1933), pp. 303-305.

[6]Shope, R. E.: Experiments on the epidemiology of pseudorabies. I. Mode of transmission of the disease in swine and its possible rôle in its spread to cattle. J. Exp. Med., lxii (1935), pp. 85-99.

[7]Shope, R. E.: Experiments on the epidemiology of pseudorabies. II. Prevalence of the disease among middle western swine and the possible rôle of rats in herd-to-herd infections. J. Exp. Med., lxii (1935), pp. 101-117.

Fig. 1. Pseudorabies in a steer. Note the effort of the animal to bite himself and the area which he has been licking.

toms were first noticed about eight hours previously and, at the time of arrival at the laboratory, consisted of a slightly increased rate of respiration, frequent licking and biting of the skin behind the left shoulder and over the left foreribs (fig. 1), and occasional spasms of the diaphragm. The rectal temperature was 103° F. Inquiry into the history of the patient revealed that six animals in the herd had within a few preceding days shown similar symptoms. The disease in each case had developed rapidly, terminating fatally within a period of 24 to 48 hours. A tentative diagnosis of pseudorabies was made and the animal held for observation.

At 9:00 a. m. the following day (February 13), the respirations of the patient had increased to 138 per minute; otherwise there seemed to be little change in the symptoms. Although hyperesthesia was evident, no tendency to attack was manifested. At 3:30 p. m., the temperature was 107.8° F. and the respirations at least 145 per minute and shallow. At this time the animal seemed disposed to lie down (on the right side) although he was still able to rise. At 5:30 p. m., the temperature had risen to 109° F. Little change in respirations was noted; however, a bloody froth was observed about the mouth and nostrils and spasms of the diaphragm were more frequent, occurring as often as five or six times each minute. It was decided to destroy the animal for autopsy at 7:30 p. m.

BLOOD EXAMINATIONS

On samples of jugular blood drawn shortly after the animal arrived at the laboratory and on the day following, it was

determined that the total leucocyte and erythrocyte counts and the differential leucocyte count were well within the normal range. The total non-protein nitrogen in the blood was found to be 41.1 mg. per 100 cc. of blood. No blood ketones were demonstrated. A hyperglycemia was present (107.35 mg. of blood sugar per 100 cc. of blood), the significance of which is not clear.

AUTOPSY FINDINGS

No significant gross lesions were observed except for a surprisingly mild pul-

Fig. 2. Pseudorabies in the rabbit and guinea pig. Note the results of biting and scratching at the point of inoculation posterior to the left elbow and the suggestion of paresis of the left front leg in the animal on the left.

monary edema and the lesions along the left side and back consisting chiefly of loss of hair, abrasion of the skin and subcutaneous hemorrhage and edema.

ANIMAL INOCULATIONS

A portion of each of the two blood samples drawn during the course of the disease was injected subcutaneously into two rabbits and two guinea pigs. All eight animals remained healthy. At autopsy of the steer, portions of the brain, the dorsolumbar spinal cord, and the urine were obtained in as aseptic a manner as possible and some of each, after the necessary preparation, was injected subcutaneously into two rabbits and two guinea pigs. All animals receiving brain suspension or urine remained healthy; however, both rabbits (997 1516) receiving the suspension of spinal cord succumbed within approximately 80 to 86 hours and one guinea pig (1547) succumbed in less than 96 hours. The second guinea pig (1520) remained healthy.

Symptoms in rabbits consisted first of biting and scratching at the point of inoculation (fig. 2). This increased until the term "mad itch" seemed well justified. Some would continue until they rolled over, exhausted, but with the teeth still grasping the skin. The result in each case was an abraded area up to several centimeters in diameter (fig. 3). Respirations increased, usually to at least twice the normal number. When not in the act of biting or scratching, the animals would lie with the nose held a little higher than usual and the head retracted and slightly inclined toward the affected side. An attitude suggesting paresis of the leg near which the virus had been injected was noted in a few instances (fig. 2). At autopsy, besides the abraded

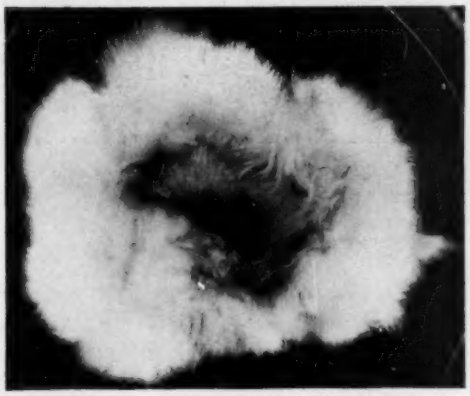

Fig. 3. The effects of biting and scratching at the point of inoculation in the rabbit with pseudorabies.

area, marked congestion and edema of the lungs were constant lesions, with subpleural petechiae and ecchymoses usually in evidence.

Using the rabbit and guinea pig-brain cortices, brain stems, and portions of the spinal cords anterior to the points of exposure, we made subcutaneous injections into nine rabbits and nine guinea pigs, with the following results: (1) The brain cortices failed to reproduce the disease in the three rabbits and three guinea pigs used, (2) the brain stems brought about death in two of three rabbits and in none of three

guinea pigs, (3) the spinal cords produced the disease and death within 90 hours in three of three rabbits and in two of three guinea pigs. By the use of more rabbit inoculations, the presence of the virus was then demonstrated in a bacteria-free filtrate (Berkefeld N) of the original steer spinal cord and in a similar filtrate made from

Fig. 4. Lesions resulting from the multiplication of the virus of pseudorabies on the chorioallantois of the developing chick embryo, 72 hours after inoculation (x 1-1/3).

the spinal cords of rabbits which died from the disease. The presence of the virus in a filtrate made from the lungs of one rabbit could not be demonstrated.

A bacteria-free (Berkefeld N) filtrate was then prepared from a composite suspension (approximately 7 to 8 per cent) of a portion of the steer spinal cord and several rabbit spinal cords and its effect on several species observed as indicated in table I.

The symptoms of the calf which succumbed following exposure paralleled very closely those of the steer from which the virus was isolated. Each showed a marked and abrupt rise in body temperature before death. The respiratory rate also continued to increase until the time of destruction. In the calf the respiratory rate increased from 108 to 208 per minute during the 5½ hours preceding destruction. Spasms of the diaphragm likewise occurred and became more frequent as the disease progressed.

CHORIOALLANTOIC INOCULATIONS

It was found possible to produce lesions on the chorioallantois of the developing chick embryo by inoculating heavily with virus-containing material after 12 days of incubation. A concentrated suspension of bacteriologically negative spinal cord tissue was suspended in tryptone broth, the suspended material allowed to settle, and the supernatant fluid used as inoculum. The resulting lesions resembled those described by Glover.[8] In about 35 to 48 hours the membranes appeared slightly more opaque than normal, and by 48 hours definite macroscopic focal lesions were present (fig. 4). These were white, slightly raised,

[8]Glover, R. E.: Cultivation of the virus of Aujeszky's disease on the chorioallantoic membrane of the developing egg. Brit. J. Exp. Path., ii (1939), pp. 150-158.

TABLE I—Results of Inoculation of Bacteria-Free Filtrate* into Various Animals

ANIMAL	DOSE (CC.)	MODE OF EXPOSURE	RESULTS
Horse	10.0	Subcutaneous	No evidence of disease
Calf	5.0	Subcutaneous	Destroyed in moribund condition 5 days after exposure; virus recovered
Pig 1	4.0	Subcutaneous	No reaction except a slight temp. rise 2d and 3d days after exposure
Pig 2	2.0	Subcutaneous	No significant reaction
Dog	2.0	Subcutaneous	Slight malaise and mild pruritus at point of inoculation, followed by recovery
Rabbit 694	1.0	Subcutaneous	Succumbed from pseudorabies within 120 hrs.
Rabbit 685	1.0	Intravenous	No visible reaction (temp. not taken)
Rabbit 1459	1.0	Intraperitoneal	Succumbed from pseudorabies within 122 hrs.
Rabbit 680	0.2	Conjunctival	No visible reaction
Rabbit 1460	0.2	Intranasal	No visible reaction

*Prepared by the use of a Berkefeld "N" filter from a composite suspension (approx. 7-8%) of a portion of the steer spinal cord and the spinal cords from several rabbits.

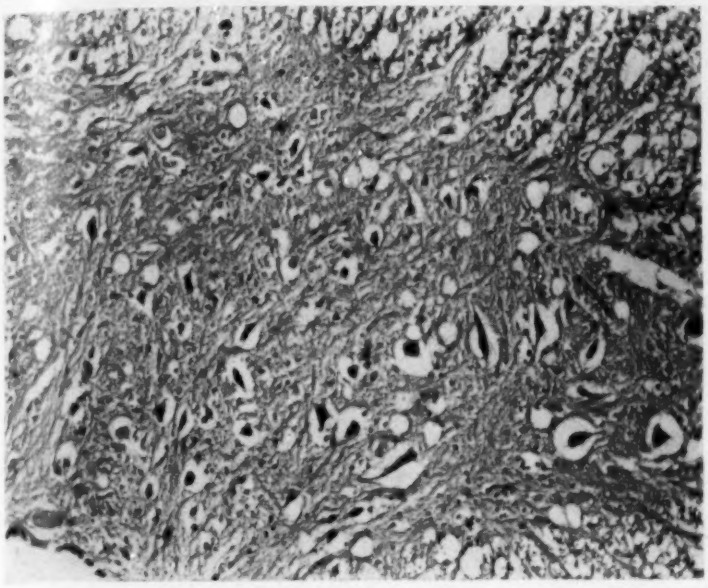

Fig. 5. Acute necrosis of neurons in the ventral horns of the spinal cord of rabbits affected with pseudorabies. A similar lesion was noted in a calf in which the disease was produced experimentally (x 107).

and varied up to approximately 0.8 mm. in diameter. Occasionally several foci were seen to be confluent.

HISTOPATHOLOGIC FINDINGS

Negri bodies were not demonstrated in smears made from the Ammon's horns of the steer. Extensive histologic examinations, in which special neuropathologic methods were used, were not undertaken. However, in sections fixed with Zenker's fluid and stained with hematoxylin and eosin, an acute reactionless necrosis of neurons of the ventral horns of the spinal cord was observed in the calf and in each of three rabbits from which sections were

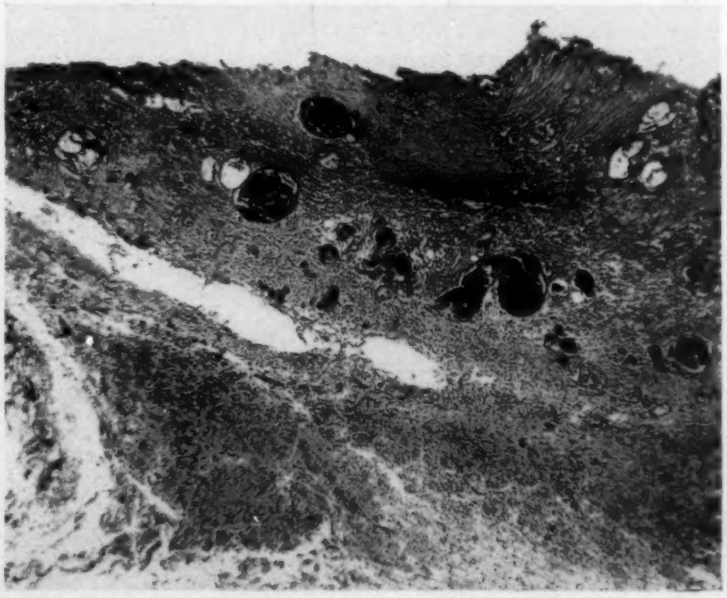

Fig. 6. Lesions produced at the site of inoculation in the rabbit. Note the erosion of epithelium and the congestion and leucocytic infiltration of the subcutis. Cell infiltration was not noted in all cases (x 33).

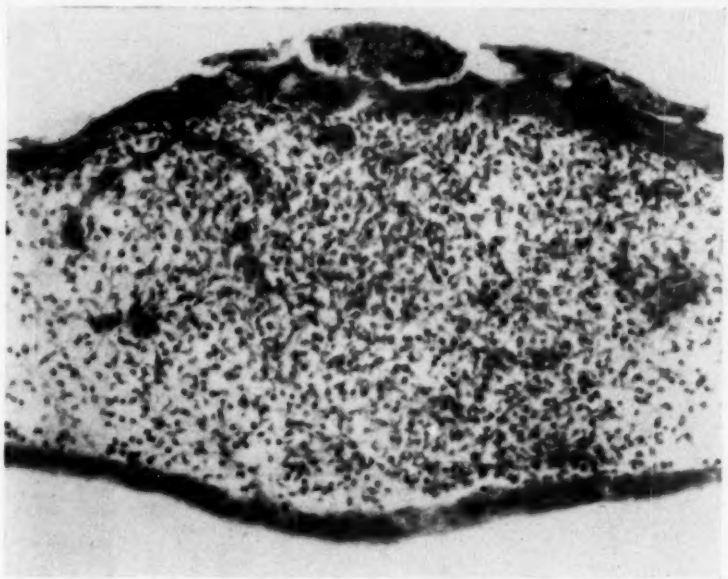

Fig. 7. Focal lesion produced in the chorioallantois of the developing chick by the virus of pseudorabies. Note thickening of the ectodermal layer with necrosis of superficial cells and thickening of the mesodermal layer due to infiltration of leucocytes and proliferation of mesodermal cells, 72 hours after inoculation (x 115).

made (fig. 5). Inclusions were not observed in the spinal cord as described by Hurst.[9] Focal hemorrhages were noted in the cerebrum of one rabbit. Lesions at the site of inoculation in the rabbit consisted of trauma and necrosis of the epithelium and marked congestion, some hemorrhage and varying degrees of leucocytic (polymorphonuclear) infiltration in the subcutis (fig. 6). Pulmonary changes in the rabbit consisted of congestion, hemorrhage and edema. Each of these varied some in degree; however, the congestion and edema were, in most instances, quite marked and diffuse while the hemorrhage tended to be focal in character.

The microscopic changes produced in the chorioallantois of the developing chick embryo were the same as those described by Glover. As he describes them, inoculation is followed by a slight focal proliferation of ectodermal cells and by edema and some infiltration of the mesoderm with acidophilic leucocytes within 24 to 36 hours. Proliferation of the ectodermal cells ceases by the 48th hour and some necrosis of the outer layers at the points of greatest thickness occurs soon thereafter. A rather marked proliferation of mesodermal cells is seen at this time beneath the areas of ectodermal thickening. The height of the reaction (as shown in fig. 7) is reached at 72 hours, soon after which regressive changes occur, leading to necrosis.

[9]Hurst, E. W.: Studies on pseudorabies. I. Histology of the disease, with a note on the symptomatology. J. Exp. Med., lviii (1933), pp. 415-433.

SUMMARY

An outbreak of pseudorabies in cattle is reported. The characteristics of the disease and its causative agent are described as they were observed during the course of laboratory diagnosis and experimentation. The attention of the reader is directed to the possibility that similar outbreaks may have been erroneously diagnosed by clinical manifestations alone and to the desirability of providing a live affected animal for laboratory diagnosis. As a control measure, the prompt removal of all swine, even though apparently healthy, from contact with the cattle is recommended.

Grass Tetany in Cattle*

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EVERY SPRING, for a number of years, a loss of cows from so-called grass tetany has occurred in central Kentucky and during the spring of 1940 the trouble was unusually prevalent. Through the coöperation of several veterinary practitioners in this locality we were able to secure blood samples from twelve cases.

REVIEW OF LITERATURE

Sjollema,[1] in 1930, first described the disease as it occurs in Holland, terming it "grass staggers." He showed that typical cases were characterized by a low serum magnesium. Dryerre[2] reported an apparently similar condition in England which he called "lactation tetany." In this state Metzger[3] described a case in a cow which had not calved for two years. Duncan, Huffman and Robinson[4] produced fatal tetany in five calves limited to a whole-milk diet. Although the calcium and inorganic phosphorus remained normal in these calves, the magnesium was low. Kruse, Orent and McCollum[5] previously produced fatal tetany in rats and dogs maintained on a diet very low in magnesium. In their studies of magnesium deficiency in rats Tufts and Greenberg[6] concluded that the severity of magnesium deficiency was increased when a high calcium content was present in the diet.

PRESENT WRITERS' OBSERVATIONS

Grass tetany, in our experience, is an often fatal disease of dairy or beef cows which occurs independently of parturition and is characterized by tetanic contraction of the limbs. The onset of the disease is sudden, appearing within a few days to several weeks after cows are turned out on young, rank grass, in early spring. Usually only one or two animals in the herd are affected. What causes the disease is not definitely known, although in the cases brought to our attention there was a history of faulty feeding during the winter. Yet, the disease does not occur until the animals have been on young, rank pasture for a short time. Sjollema,[1] from his extensive study of the disease, concluded that the primary cause of "grass staggers" is connected with intestinal intoxication which may be the cause or the effect of the upset in mineral metabolism. The practice in Holland, of feeding little hay or hay of poor mineral composition and large quantities of concentrates, he considers a predisposing cause. Of the twelve cases we have studied eleven occurred during the winter when the animals were almost entirely on poor quality hay. Apparently some animals possess a more delicately balanced mineral metabolism which is unable to cope with conditions which other members of the herd successfully meet.

Symptoms.—The first symptoms usually are not observed by the owner and it is not until the animal is down with tetanic convulsions that the veterinarian is called. Early symptoms of the disease include twitching of the brachial muscles, a tendency to graze apart from the herd, nervousness and an anxious expression. In the late stages the animal suddenly becomes prostrate and develops what most owners refer to as a "fit." This syndrome consists of tetanic contractions of the limbs, grind-

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*From the Department of Animal Pathology, Kentucky Agricultural Experiment Station; the investigation reported herein was conducted in connection with a project of the Station.

[1]Sjollema, B.: On the nature and therapy of grass staggers. Vet. Rec., x (1930), pp. 425-430, 450-453.

[2]Dryerre, H.: Lactation tetany. Vet. Rec., xii (1932), p. 1163.

[3]Metzger, H. J.: A case of tetany with hypomagnesemia in a dairy cow. Cornell Vet., xxvi (1936), pp. 353-356.

[4]Duncan, C. H., Huffmann, C. F., and Robinson, C. S.: Magnesium studies in calves, I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem., xviii (1935), pp. 35-44.

[5]Kruse, H. D., Orent, Else R., and McCollum, E. V.: Studies on magnesium deficiency in animals. III. Chemical changes in the blood following magnesium deprivation. J. Biol. Chem., c (1933), pp. 603-644.

[6]Tufts, Elma V., and Greenberg, D. M.: The biochemistry of magnesium deficiency. I. The minimum magnesium requirement for growth, gestation, and lactation and the effect of the dietary calcium level thereon. J. Biol. Chem., cxxii (1938), pp. 715-726.

ing of the teeth, frothing at the mouth, trismus, bellowing, retraction of the head with death finally resulting from severe tonic and clonic convulsions. Despite treatment with calcium salts, the mortality in the cases studied was over 50 per cent. The prognosis varied directly with the post-parturient interval; that is, the longer the interval between the date of calving and the onset of the disease, the greater was the percentage of mortality.

Case 1.—A 5-year-old Holstein cow which calved eight weeks previously was found lying in the pasture on the morning of April 10, 1940. This cow was fed on ensilage and very little hay during the winter. In lieu of a urine sample, a portion of milk was tested by the Ross test and found to be negative for acetone. Treatment consisted of the intravenous injection of 250 cc. of 20 per cent glucose followed by 50 cc. of a 10 per cent magnesium sulfate solution. After this treatment the convulsions ceased, leaving the animal in a semicomatose condition. The owner later reported that the animal died at 4 p.m. of the same day. Analysis of blood drawn previous to treatment showed that the proportions of magnesium, inorganic phosphorus and calcium in the serum were distinctly below normal, while non-protein and protein nitrogen were normal.

Case 2.—A 4-year-old Holstein cow was treated for milk fever on February 8, 1940. On February 9, she received medication for acute acetonemia. Two days later she was found to be in an apparently moribund state. The acetonemia treatment was repeated and she made a satisfactory recovery. On April 10, she developed typical grass tetany and was given 500 cc. of a 20 per cent solution of calcium gluconate. Her condition was immediately improved. On April 28, this cow was treated again for grass tetany, at which time she regained her feet, but suddenly became prostrate and died. A sample of serum collected April 10, analyzed in this laboratory, was found to be markedly low in magnesium and inorganic phosphorus. Serum calcium, although subnormal, was not as low as is found in milk fever. Serum protein was found to be above normal.

Case 3.—A limited history was received with this sample, but as samples were requested only from cases showing typical tetany, we may conclude that the usual nervous syndrome was present. This animal was a 5-year-old Shorthorn cow which calved six months previously and was rebred. She was fed on grass only. Treatment with calcium gluconate failed. A marked hypomagnesemia was the outstanding serologic change observed on chemical examination.

Case 4.—This case and the following one were from a herd of beef cattle on early spring pasture. On May 20, 1940, four cows presented typical grass-tetany symptoms. Unfortunately, samples were collected from only two of these cases. Their diet had consisted solely of grass for the previous two weeks, prior to which they received lespedeza hay along with very small amounts of concentrates.

This case was a 6-year-old Hereford beef cow which calved 5 weeks previously. Samples of oxalated and whole blood were obtained prior to treatment.

Treatment included the intravenous injection of 50 cc. of 10 per cent magnesium sulfate and 500 cc. of 20 per cent calcium gluconate. Convulsions ceased and prompt improvement was noticed. Two hours later the animal suffered a relapse and died. The serum magnesium was approximately one twentieth of the normal value. A definite hypocalcemia and hypophosphatemia also were observed.

Case 5.—This was a 7-year-old Hereford beef cow from the same herd as case 4. She calved about three weeks before the onset of the grass-tetany syndrome and appeared to be normal until she was found lying in the pasture with three other cases. Treatment consisted of the intravenous administration of 500 cc. of 20 per cent calcium gluconate. Attempts to get the animal on her feet were unsuccessful at the time, but later in the day she got up of her own accord and made an uneventful recovery. The fate of the other two cases is unknown, as we received no further information from the practitioner who treated them. Examination of the blood revealed a magnesium content of 0.1 mg. per cent accompanied by a low calcium of 5.8 mg. per cent. We were surprised to hear later that this animal recovered. From table I it can be readily seen that the normal inorganic phosphorus value in this case is the most noticeable variation.

Case 6.—This was a 5-year-old Hereford cow which calved four weeks previously. She was fed lespedeza hay with no grain during the previous winter. For the past month she was pastured on young, rank grass. On the morning of April 23, she was found lying in the pasture having convulsions. To quiet the animal, one ounce of cannabis indica was given intravenously, followed by a slow intravenous injection of 500 cc. of cal-dextro. Although generalized muscle tetany disappeared, she was unable to get to her feet. One-half hour later a 1-oz. capsule of chloral hydrate was given *per os*. That evening the hired man told the veterinarian that the cow was doing fine, but the next day she was found dead in the pasture. Serum magnesium and calcium were low, as usual, with the inorganic phosphorus

TABLE I—Composition of Blood and Serum in Reported Cases

Case Number	Whole Blood			Serum			
	Glucose (mg. per 100 cc.)	Total Non-protein Nitrogen (mg. per 100 cc.)	Cell Volume (Per Cent)	Protein (Per Cent)	Calcium (mg. per 100 cc.)	Inorganic Phosphorus (mg. per 100 cc.)	Magnesium (mg. per 100 cc.)
1	N. E.*	N. E.	58.0	7.34	7.60	2.40	.86
2	N. E.	N. E.	N. E.	8.56	6.85	2.28	1.14
3	N. E.	N. E.	N. E.	6.48	6.80	4.85	.59
4	52.5	30.6	43.0	5.72	5.90	2.38	.10
5	63.0	37.5	42.0	6.18	5.80	4.95	.10
6	140.0	33.0	33.0	6.97	6.30	4.60	.86
7	75.0	30.6	43.0	7.31	7.30	4.40	1.68
8	N. E.	N. E.	N. E.	N. E.	5.80	1.50	2.46
9	N. E.	N. E.	N. E.	6.34	8.35	1.33	.69
10	N. E.	N. E.	N. E.	N. E.	8.50	4.05	.46
11	106.7	30.3	N. E.	N. E.	4.90	5.50	1.18
12	N. E.	N. E.	N. E.	N. E.	5.50	2.55	.62
Average	87.44	31.65	43.8	6.86	6.63	3.40	.903

*N. E.=Not estimated.

normal. A marked hyperglucemia (140 mg. per 100 cc. of blood) was present; this high glucose level might well be due to the convulsion at the time the blood samples were drawn.

Case 7.—This was a 9-year-old grade Holstein cow which calved five weeks before the onset of the disease. She was in poor physical condition, having received only pasture grass and a small quantity of clover hay for the past three weeks. The owner stated that this cow was apparently normal in the evening of April 22, but at 5:00 the next morning was found prostrated in the pasture and could not get up. At 1:00 p. m. the owner inflated the udder, without success. A urine sample, obtained by catheterization, was negative for acetone, but contained 75 mg. of albumin per 100 cc. Injection of 300 cc. of cal-dextro intravenously and 200 cc. subcutaneously resulted in immediate recovery. Within a few minutes she regained control of her feet and, with the exception of slight trembling in the brachial region, appeared perfectly normal. Subsequent reports indicated that relapses occurred which were treated with additional doses of cal-dextro, a total of 2,500 cc. having been used before recovery was complete. A relatively high magnesium content, when compared with the average value for these cases, was obtained in this instance. Calcium dropped about 20 per cent below normal, while the inorganic phosphorus remained at a proper level.

Case 8.—A 9-year-old Guernsey was found lying in the pasture. Some doubt existed as to whether we should classify this case as delayed milk fever or as grass tetany. This cow calved two weeks previously but suddenly became prostrate, exhibiting the typical grass-tetany syndrome. Her diet was much better than was usual in the cases in this locality. Rations in use at that time included a 16 per cent protein concentrate, alfalfa hay and cornstalks. As the attending veterinarian has had a great deal of experience with this disease, his diagnosis was considered reliable. On examination of the serum the magnesium content was slightly above normal. Such incidences have also been reported by Sjollema in which the magnesium content remained normal. The veterinarian treated this animal with 500 cc. of cal-dextro intravenously and a pound of magnesium sulfate *per os*, and advised the owner to leave her in the barn for ten days. This case was reported as having made a satisfactory recovery. Investigation of the serum revealed a normal magnesium content in conjunction with a definitely low calcium and phosphorus level.

Case 9.—This was a 7-year-old Holstein cow with a week-old calf. Her winter rations consisted of cornstalks, poor hay, unshelled corn and some lespedeza hay. The general condition of the animal was poor and the year previously she was treated for the same condition. Treatment consisted of 500 cc. of cal-dextro intravenously, a magnesium sulfate physic and advice to the owner to put the animal inside for ten days. This case made an uneventful recovery. Subnormal values were recorded for magnesium, calcium and inorganic phosphorus, such values being especially low for magnesium and inorganic phosphorus.

Case 10.—An 8-year-old Shorthorn cow calved eight months previously and was barren. This

animal had received no dry feed for two months, being pastured on young bluegrass. As is often the case, the condition of the animal was poor and the symptoms exhibited were severe. Treatment included 500 cc. of cal-dextro intravenously and 1 oz. of chloral hydrate *per os*. Dry feed was recommended. This animal made a temporary recovery for one week and then suffered a relapse followed by death. Serum analysis revealed a definite hypomagnesemia, while the calcium and inorganic phosphorus were almost normal.

Case 11.—A 5-year-old grade Hereford cow calved seven months previously and was pregnant at the time of this observation. During the past winter this cow was fed on ensilage and lespedeza hay and for the past three weeks was on grass only. The animal became prostrate 24 hours previously, but got up after having a "fit," as the owner expressed it. The following day she was again prostrated and at the time we saw the animal she was lying on her side, with feet extended but not threshing with them as is usually the case. Following the intravenous injection of 500 cc. of 20 per cent glucose and 500 cc. of cal-dextro, attempts to force the animal to her feet were unsuccessful. Death occurred 24 hours later following a typical convulsion. Chemical analysis of the blood showed an unusually low serum calcium, a 50 per cent reduction of magnesium with the inorganic phosphorus within the normal range.

Case 12.—A 6-year-old Jersey cow calved two weeks previously and had been on young pasture for one month. As no description of the symptoms was sent with the blood sample, we can only assume that it was a typical case. An uneventful recovery followed the administration of 500 cc. of calcium gluconate intravenously, 0.5 oz. of chloral hydrate *per os* and advice to the owner to put the animal on dry feed. From table I it can readily be seen that the low results obtained for magnesium, calcium and inorganic phosphorus would confirm the veterinarian's diagnosis.

DISCUSSION OF BLOOD ANALYSES

Magnesium.—The average serum magnesium value given by Sjollema for normal cows was 1.66 mg. per cent and .46 mg. per cent for cows with grass tetany, which is considerably lower than our value of 2.32 mg. per cent for normal pregnant cows and 0.9 mg. per cent in grass-tetany cases. In their work on tetany in calves Duncan, Huffman and Robinson[4] reported a plasma magnesium range of 2.25-2.75 mg. per cent for normal calves and 1.2-1.6 mg. per cent for calves suffering from low-magnesium tetany. Dogs maintained for five weeks on a magnesium-deficient diet by Kruse, Orent and McCollum showed a level between 0.5 and 0.9 mg. per cent compared with a level in control animals between 2.1 and 2.3 mg. per cent. From these observations the following conclusions might justly be made, namely, that the bovine species is susceptible to magnesium deficiency and that in grass tetany magnesium values comparable with experimental results are obtained. Whether this low magnesium observed in grass tetany is due to a dietary deficiency we are unable to answer, although Sjollema is of the opinion that such is not the case.

Calcium.—Calcium values in grass tetany differ significantly from experimental cases of magnesium tetany. Serum calcium values for normal cows range between 9 and 12 mg. per cent (Hayden). In a series of 15 normal cows, one month prior to parturition, we obtained an average value of 10.99 mg. per cent. Grass tetany cases ranged from 4.9 to 8.5 mg. per cent with an average of 6.63 mg. per cent of calcium, which closely approximates Sjollema's 6.65 mg. per cent representative of 55 cases. In strict magnesium deficiency, calcium and inorganic phosphorus remain normal. This tendency towards lowered calcium as shown by our results and those of Sjollema resembles a combined calcium-magnesium deficiency in which a terminal drop is observed in the serum calcium.[7] That the calcium content in cases of this type may vary with geographic distribution is well illustrated by the relatively high average of 9.3 mg. demonstrated by Dryerre,[2] who studied the disease in the British Isles.

Inorganic Phosphorus.—Usually the calcium and inorganic phosphorus of the blood vary in an inverse manner. Thus, a decrease in the concentration of calcium might lead one to expect an increase in the level of the inorganic phosphorus. In our experience this relationship is not maintained either in milk fever or grass tetany.

[7] Day, Harry G., Kruse, H. D., and McCollum, E. V.: Studies on magnesium deficiency in animals. VII. The effects of magnesium deprivation with a superimposed calcium deficiency on the animal body, as revealed by symptomatology and blood changes. J. Biol. Chem., cxii (1935), pp. 337-359.

TABLE II—Comparison of Average Values Obtained by Different Investigators*

INVESTIGATOR	CALCIUM	INORG. PHOS.	MAGNESIUM	CASES
Sjollema	6.65	4.33	.455	55
Dryerre	9.30	4.54	.390	?
Authors	6.63	3.40	.903	12
Metzger	7.50	2.30	.145	1

*All values expressed as milligrams per 100 cc. of serum.

Diets deficient in magnesium alone do not result in a lowering of the phosphorus content of the blood. However, when calcium deficiency is superimposed on a magnesium deficiency, a terminal drop occurs in the serum inorganic phosphorus.[7] The average serum inorganic phosphorus as given by Sjollema for normal cows was 4.57 mg. per cent, and 4.53 mg. per cent for cows with grass tetany. In "lactation tetany" Dryerre[2] likewise failed to observe any significant decrease in the serum inorganic phosphorus. Hayden[8] presented evidence that little variation exists in the serum inorganic phosphorus between nonpregnant cows and cows at or following parturition. Working with normal dairy cows one month prior to parturition we found an average of 5.48 mg. per cent for serum inorganic phosphorus. In contrast to this normal level we obtained an average of 3.39 mg. per cent in twelve cases of grass tetany. In the single case reported by Metzger[3] from Kentucky, a serum inorganic phosphorus value of 2.53 mg. per cent was found prior to treatment and 5.43 mg. per cent three weeks after recovery.

Miscellaneous Findings.—In two instances the blood-sugar level was abnormally increased. As the respirations are severely inhibited during the convulsions, the partial asphyxia may account for this increase in the blood sugar.

The terminal rise in total non-protein nitrogen which holds true in magnesium deficiency[5] and calcium-magnesium deficiency[7] was not observed in grass-tetany cases. Serum protein likewise failed to show any appreciable variation from the normal.

Cell volume was determined in five cases on blood containing 0.2 per cent potassium oxalate. Three of the cases were normal, one was high and the remaining case might be called a low normal.

[8]Hayden, C. E.: Changes in the blood of the cow at parturition. Cornell Vet., xxviii (1938), pp. 152-160.

Standardization of Stained Brucella Abortus Antigen for the Whole-Blood Test*

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IN 1935 Welch and Marsh[1] reported the development of a whole-blood field agglutination test for Bang's disease in range cattle. The antigen described was prepared according to the method of Donham and Fitch,[2] with the exception that only one strain of the organism was used, and with the addition of 5 per cent of a saturated aqueous solution of methyl violet. The reason for adding the violet stain to the antigen was to make the reaction readable against the red background of the blood, but the stained antigen proved so much more satisfactory for use with serum, as well as whole blood, that it is now used exclusively in this laboratory for all plate testing.

It was found that the antigen prepared by this method was slightly too sensitive. Therefore, the addition of 0.4 per cent gelatin, as recommended by Donham and Fitch, was eliminated. Another change in the preparation of the antigen was the use of five strains of the organism rather than only one.

After these changes were made, it was found that the antigens were not constant in sensitivity. Therefore, a number of experimental antigens were prepared in an attempt to standardize the method of preparation in order to insure a constant sensitivity. Roepke and Fitch[3] showed that an important factor in the variation in sensitivity of *Brucella abortus* antigens is the soluble agar concentration, and that this effect can be minimized by washing the cultures from the agar medium with large amounts of saline solution and then centrifuging to remove the substances absorbed from the medium. Therefore, this portion of the method of preparing the antigen at this laboratory has been changed to correspond to the method used in the laboratory of the Division of Veterinary Medicine, Minnesota Agricultural Experiment Station. The cultures are washed from the medium with sufficient phenolized salt solution to produce approximately a 1.5 per cent suspension of cells. This suspension is filtered through a thin film of cotton and heated in flowing steam for 2½ hours. It is again filtered through a thin film of cotton, and centrifuged. The sediment is washed by resuspending in phenolized saline and removing the saline solution by centrifugation. The cells are then taken up in sufficient phenolized saline solution to make an 11 per cent suspension.

Roepke and Fitch stated that antigens prepared in this manner "show titers which average approximately one-half dilution (an incomplete agglutination) lower than the tube antigen. The presence of 0.4 per cent gelatin in the plate antigen seems to give a uniform increase in sensitivity and results in antigens which give titers comparable to those obtained with the tube antigen." As stated earlier in this paper, it was found in this laboratory that the addition of gelatin to the stained antigen made it too sensitive. Roepke, in a personal communication, suggested that the concentrated stain which is added to the antigen for the whole-blood test acted in a similar manner to the gelatin in increasing sensitivity. He also suggested that variations in sensitivity might be attributed to variations in depth of staining and in the

*From the Montana Veterinary Research Laboratory, Montana Livestock Sanitary Board and Agricultural Experiment Station coöperating; paper No. 142, journal series, Montana State College, Agricultural Experiment Station.

[1]Welch, H., and Marsh, H.: A whole-blood field agglutination test for Bang's disease in range cattle. J.A.V.M.A., lxxxvi (1935), pp. 493-507.

[2]Donham, C. R., and Fitch, C. P.: A method for the preparation of antigen for the rapid agglutination test for Bang's disease with a technic for conducting such a test. J.A.V.M.A., lxxxii (1933), pp. 913-921.

[3]Roepke, M. H., and Fitch, C. P.: J. Inf. Dis., lxvi (1940), p. 17.

TABLE I—Comparison of Methyl Violet 6B with Crystal Violet as to Sensitivity of Antigen

Serum	Stain	Dilutions			
		.04 cc.	.02 cc.	.01 cc.	.005 cc.
1	Methyl violet 6B	++	++	+	—
	Crystal violet	++	++	+	—
2	Methyl violet 6B	++	++	±	—
	Crystal violet	++	++	+	—
3	Methyl violet 6B	++	++	++	+
	Crystal violet	++	++	++	±

++ = Complete agglutination.
+ = Partial agglutination.
± = Slight agglutination.

TABLE II—Comparison of Methyl Violet 6B with Crystal Violet as to Sensitivity and Readability of Antigen, Using Whole Blood

Blood	Stain	Dilutions					Readability
		.04 cc.	.03 cc.	.02 cc.	.01 cc.	.005 cc.	
1	Methyl violet 6B	++	++	++	±	—	Less
	Crystal violet	++	++	++	—	—	More
2	Methyl violet 6B	++	++	++	—	—	Less
	Crystal violet	++	++	++	±	—	More
3	Methyl violet 6B	++	++	++	+	—	Less
	Crystal violet	++	++	++	+	—	More

TABLE III—Effect of Concentration of Stain on Sensitivity of Antigen

Serum	Dye Concentration	Dilutions			
		.04 cc.	.02 cc.	.01 cc.	.005 cc.
1	10% of 1.5% solution	++	++	±	—
	5% of 1.5%	++	++	+	—
	3% of 1.5%	++	++	±	—
	1% of 1.5%	+	+	—	—
2	10% of 1.5%	++	++	++	—
	5% of 1.5%	++	++	++	+
	3% of 1.5%	++	++	++	+
	1% of 1.5%	++	++	++	+
3	5% of 1.5%	++	++	+	±
	3% of 1.5%	++	++	±	—
	1% of 1.5%	++	±	—	—
4	5% of 1.5%	++	++	++	—
	3% of 1.5%	++	++	+	—
	1% of 1.5%	++	++	±	—

dye itself. Therefore, a number of antigens were prepared in which the method of adding the stain, the particular dye used, and the concentration, were varied.

In the original stained antigens used in this laboratory, the staining material was added as the final step in the preparation of the antigen, a small amount of a concentrated aqueous solution being used. Roepke suggested that the use of a concentrated (saturated) solution could be avoided by making the final suspension of the cells in a phenol-saline solution containing a definite amount of stain sufficient to give the desired sensitivity and readability. An attempt was made to prepare solutions of crystal violet in .85 per cent saline solution and in phenolized saline solution. It was impossible to make a stable solution of crystal violet in either the saline or phenol-saline solutions. This was probably due to "salting out" of the crystal violet by the sodium chloride in the solutions. The phenol also had some effect in preventing the preparation of a stable solution. However, when 5 per cent of the saturated aqueous solution of the dye was added to the 11 per cent bacterial suspension in phenolized saline solution, there was no crystallization of the dye. Therefore, the method of adding the dye which was adopted, was the original method of adding it in saturated (1.5 per cent) aqueous solution. It was found necessary to use a freshly prepared and filtered solution.

The dye recommended in our original publication was methyl violet 6B (Coleman and Bell), but crystal violet (Coleman and Bell) also has been used. The results of a comparison of the sensitivity and readability of antigens stained with these two dyes are shown in tables I and II. An antigen which has been found to correspond to the standard tube antigen in sensitivity was divided into two lots, one being stained with methyl violet 6B and the other with crystal violet. Plate agglutination tests comparing the two antigens for sensitivity were set up with three serums of intermediate titer, as shown in table I. The results indicate that these two stains are alike in their effect on the sensitivity of the antigen.

Table II shows a comparison of both sensitivity and readability of the same two antigens used in the experiment recorded in table I, when used with whole blood from the same three cattle from which the serums used in the previous test were taken. An additional dilution was used in this experiment, as .03 cc. of whole blood corresponds in reaction with the antigen to .02 cc. of serum. This table again shows no difference in sensitivity, but with the whole blood, reactions were more easily read in the case of the antigen stained with crystal violet. Therefore, crystal violet (Coleman and Bell) has been adopted as the dye to be used.

The third factor to be considered in determining the effect of the stain on sensitivity is the concentration of the dye. A plate antigen, which corresponded to the standard tube antigen in sensitivity when 5 per cent of the dye solution was added, was divided into four lots. To one lot was added 10 per cent of a 1.5 per cent aqueous solution of the dye, to the second lot 5 per cent was added, to the third lot 3 per cent was added, and to the fourth lot 1 per cent was added. A comparative test was set up in which four serums of intermediate titer were used, as shown in table III. The results tabulated in table III show that raising the concentration of the dye above 5 per cent of a 1.5 per cent solution did not increase the sensitivity of the antigen, but that lowering the concentration below that point did decrease the sensitivity below the desired point. The conclusion was, therefore, that the optimum concentration of dye was 5 per cent of a 1.5 per cent aqueous solution, or a final concentration of .075 per cent, and that the effect of this concentration of crystal violet on sensitivity corresponds to the effect of 0.4 per cent of gelatin.

Since the completion of these experiments, three lots of antigen have been prepared and stained by the methods indicated here as giving the best results, and there has been almost no variation in sensitivity. One of these antigens was checked against

the tube test in testing 468 cattle in one range herd, with almost completely corresponding results. In this herd there were no reactions to the tube test at a higher dilution than 1:25. Five of the blood samples showed partial agglutination at this dilution in both tests. Traces of agglutination were recorded in four other samples in the field test which were negative in the tube test, while two samples which agglutinated slightly in the tubes at 1:25 were recorded in the field test as negative. This herd test is of particular interest when compared with the last preceding annual test of the same herd. At that time the stained field antigen was somewhat too sensitive, resulting in partial agglutination in 36 blood samples which showed no agglutination at 1:25 in the tube test.

SUMMARY

It has been found possible to eliminate significant variations in sensitivity of the stained antigen for the whole-blood agglutination test for Bang's disease (1) by using five strains of *Brucella abortus* of known antigenicity; (2) by washing the culture growth from the medium with large amounts of phenolized saline solution, and washing the cells once in phenolized saline before making the final suspension, as recommended by Roepke and Fitch; and (3) by staining the antigen by the addition of 5 per cent of a freshly prepared and filtered 1.5 per cent (saturated) aqueous solution of crystal violet (Coleman and Bell) as the last step in the preparation of the antigen. This concentration of dye has the same effect on sensitivity as 0.4 per cent gelatin.

Experimental Work upon Recent Outbreaks of Abortion in Ewes*

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EXCESSIVE LOSSES from abortion in ewes are not common, but each year for several years outbreaks in Wyoming have been brought to our attention and have been investigated. Previous to 1937, we received few inquiries about abortion in ewes and we heard of none of any consequence. Although quite a few reports of it have since come to us, we are not certain that the disease is spreading or is on the increase. It is possible that it existed to the same extent previously but was not called to our attention. The condition is important to the veterinarian, therefore, because in the spring he may encounter it in a high percentage of the ewes in either a range flock or a farm flock.

The owner, naturally, is suspicious of any roughage he has fed since the time the bucks were turned in. He is especially suspicious of such things as mouldy beans that have fallen from the vines and thus been left in the field he pastures. He is also often suspicious of frosted beet tops, straw stacks in the pasture, bean hulls and even the alfalfa hay. In case the ewes have not been getting any of these types of feed, the owner may suspect some type of weed or plant the animals have eaten on the range, in the pasture, or in native hay. It is our belief that the possibility that feed causes a great amount of abortion in a flock of ewes is as remote as the possibility of its causing considerable abortion in a herd of cattle. Baker and Stone[1] conducted feeding tests upon mouldy bean pods to determine if they would produce abortion in ewes. Of four ewes fed large quantities of them, none aborted.

No doubt a lack of feed to the extent that malnutrition and severe emaciation develop may result in loss from abortion. The veterinarian can recognize such cases by the history and by the general condition of the animals in the flock. It is quite generally admitted that old ewes often abort from no apparent cause other than old age. In a flock where a high percentage of the ewes are quite old, considerable abortion may be encountered. It is a good policy, therefore, in making an investigation to examine the mouths of a number of ewes that have aborted because the finding of young ewes that have aborted will help in making a diagnosis.

In addition to the many questions concerning the feed as a factor in the trouble, the owner will have numerous other ones. If he was using new bucks on ewes that had no trouble the previous year, he will be suspicious of the bucks. He will want to know if there is a blood test to determine which animals are diseased; also, if ewes that abort this year will carry the disease over to next year and abort again. The permanent infection of his range or premises or, at least, a carry-over of the infection to another year is something that is of great concern to him. If he is informed that the disease is probably due to a microörganism rather than to the feed, he wants to know how long it is from the time the ewes are infected until they abort, so that he can figure out where the ewes were at that time and keep away from that watering place, range, pasture or feed. Bang's disease as a possible cause will be asked about. And above all, he will want to know if an aborting ewe can transmit the disease to other ewes.

In endeavoring to determine some of the answers to these questions, investigations have been made upon all severe abortion losses in ewes that have been brought to our attention during the past four years. An effort had been made to check up on the flocks the following year. The results of

*From the Wyoming Agricultural Experiment Station.

[1]Baker, D. W., and Stone, W. S.: A study of *Spirillum ovis* infection in a group of ewes maintained under observation for two successive lambing seasons. Cornell Vet., xxxix (1939), p. 32.

previous years have been reported[2] and will not be repeated, but our work and findings of this year's investigation will be given. These have consisted of five outbreaks in different areas or parts of Wyoming. Two of these were range flocks, two were farm flocks and one was a "semi-range" flock.

Flock 1 consisted of 1,702 ewes due to start lambing March 16. On February 3, they started aborting from five to seven lambs a day. By February 24, they were aborting from seven to twelve a day. Four hundred and fifty ewes aborted in this flock of which only three died. Flock 2 contained 1,015 ewes due to start lambing the middle of April. When materials were submitted to us on April 8, 60 ewes had aborted. Ten of them aborted on the night of April 7. A total of 100 aborted by April 15, and 25 ewes died. Flock 3 had 400 ewes of which 50 had aborted by April 25, when called to our attention. Flock 4 contained 275 ewes due to start lambing March 20. On February 15, abortions started at the rate of one to four a day. By March 12, 70 had aborted and 25 of the ewes had died. On March 27, 125 of these ewes had died, many of which had not aborted. The ewes were thin, had no milk for the lambs and had not had enough feed for a long time. Flock 5 was a small farm flock of 87 ewes in which quite a few lambs had been aborted one to two months before time. The first abortion was on March 28. On April 10, eleven had aborted, and on May 11, a total of 22 had been lost. None of the ewes died.

Bacteriologic examinations were made upon aborted lambs from all these flocks. Smears made direct from the tissues and stained did not give results from which a causative agent could be satisfactorily determined. Cultural work consisted in the inoculation of cystine-veal-infusion-dextrose-agar slants which were then sealed. Materials most commonly used for the inoculations were the contents of the abomasum, the blood, liver and spleen of the aborted lambs. After incubation for three to seven days, a Vibrio was consistently found in lambs from flocks 1, 2 and 5. Usually this organism was found in pure culture. Cultures from the abomasum were positive in all cases from the three flocks. In some instances the blood, liver, spleen and peritoneal fluid also gave positive cultural findings of the Vibrio. The vaginal discharges and placenta were not found to be satisfactory for isolation of the organism in routine work because of excessive contamination.

The organism has previously been described as a Vibrio and as a Spirillum. In our experience it shows a preponderance of vibrionic forms in recently isolated cultures and in rapidly growing cultures, however. But in older cultures it assumes spiral shapes and the vibrionic forms are much fewer in number. As a culture ages, the cells secrete a large amount of a mucilaginous substance and become difficult to stain. In the course of a few days to a week the cells are no longer demonstrable in a culture by staining methods and only the mucilaginous substance seems to remain. Viability of the culture disappears with the production of this material and cultures have to be transferred at about five-day intervals to keep them alive.

The organism is Gram negative, highly motile, non-spore-forming and without capsules. In young cultures it stains readily. In shake cultures it grows first in a layer about 1/16 of an inch below the surface before spreading to the surface. It does not grow below this level, indicating that the organism is microaerophilic and facultatively aerobic.

By transferring 0.1-cc. amounts, it has been possible to maintain the organism in cystine-veal-infusion broth. The addition of various carbohydrates does not change the amount of growth and the carbohydrates are not fermented.

A Vibrio has been isolated from aborted lambs by a number of workers. It was first reported in England in 1912 by Mc-

[2] Lee, A. M., and Scrivner, L. H.: An investigation of severe losses from abortion in ewes. J.A.V.M.A., xcii (1938), p. 68.

TABLE I—Summary of Tests on Sera of Seven Ewes

Sample No.	Dilutions				
	1:25	1:50	1:100	1:200	1:400
1	+	+	+	+	I
2	+	I	—	—	—
3	+	+	+	+	I
4	+	+	I	—	—
5	—	—	—	—	—
6	+	+	+	+	+
7	+	+	I	—	—

Fadyean and Stockman.[3] Carpenter[4] encountered it in New York in 1918. Welch and Marsh[5] in 1924 reported work upon it in Montana, Graham and Thorp[6] in Illinois in 1929, and Gilman[7] in New York in 1934.*

Considerable technical difficulties have been encountered in attempts to make a suitable antigen from the Vibrio due to its slow, scant growth and the production of large amounts of mucin or gummy-like substances. However, enough satisfactory antigen was made to test the sera of seven ewes that had aborted in a flock in which the Vibrio was found as the causative agent. Table I summarizes these tests.

All samples were negative to the Bang's test. Bruce[8] in 1930 found that a number of ewes in an aborting flock in Canada were positive to the Bang's test in dilutions as high as 1:5,000. Graham and Thorp[6] in Illinois found one field case of vibrionic abortion positive to the agglutination test, using the Vibrio as the antigen. Welch and Marsh[5] of Montana in 1924 injected three ewes intravenously with the Vibrio. All three aborted and the sera of two of them shortly after aborting was positive in the 1:50 dilution and incomplete in the 1:100 dilution. Although we have obtained a high agglutination titer on some of the sera of aborting ewes, the test at present does not offer a practical means of determining the infected individuals or even of diagnosing the disease in a flock.

The trouble usually is encountered late in the season when most ewes are soon due to lamb, which may account for the fact that few attempts have been made in the United States to reproduce the disease with the Vibrio. Where successful results have been obtained, intravenous inoculation was commonly used. If one wishes to determine the effects of the oral administration of such materials as pure cultures of the Vibrio, the vaginal discharges of aborting ewes and the fetal fluids, the ewes should be far enough away from lambing to allow time for infection to be produced. The organism appears to lose its virulence on artificial media rather soon, and this is a handicap to work in reproduction. Welch and Marsh[5] placed five pregnant ewes on experiment in connection with this phase of the work. Two were given the organism orally and did not abort. Three were injected intravenously and they all aborted.

Graham and Thorpe[6] found that the injection of the Vibrio did not cause pregnant guinea pigs, rabbits or sows to abort. Stockman and McFadyean[3] also found that the organism would not produce abortion in guinea pigs and rabbits and that it was not pathogenic for them. By a combination of injections with fresh material containing the living Vibrio, they succeeded in producing abortion and again finding the Vibrio in 18 out of 68 pregnant ewes. The method of infection consisted in oral administration, intravenous inoculation or injection into the wall of the vagina. In some cases two or all of these channels were used, and in some cases large amounts were given at different intervals after conception. Just how many of these received the organism

*After this article was received for publication, another report dealing with vibrionic abortion in sheep was received and published by the American Veterinary Medical Association. See J.A.V.M.A., xcvii (Nov. 1940), pp. 452-453.

[3]McFadyean, J., and Stockman, S.: A Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire into Epizoötic Abortion. Part III and Appendix to Part III, Abortion in Sheep. (Wyman and Sons, Ltd., London, 1912.)

[4]Carpenter, C. M.: Researches upon Spirillum associated with abortion in ewes. Ann. Rpt. New York State Vet. Coll., 1918-1919 (1920).

[5]Welch, H., and Marsh, H.: Vibrionic abortion in sheep. J.A.V.M.A., lxv (1924), p. 203.

[6]Graham, R., and Thorp, F. Jr.: Vibrionic abortion in sheep. J.A.V.M.A., lxxvi (1930), p. 568.

[7]Gilman, H.: Vibrionic abortion in sheep. Cornell Vet., xxiv (1934), p. 207.

[8]Bruce, E. A.: Brucelliasis in sheep. J.A.V.M.A., lxxvi (1930), p. 841.

TABLE II—Results of Experimental Infection

Ewe No.	Method of Infection	Date	Effect	Date	Lamb	Incubation Period	Vibrio Isolated
4	Pooled fetal fluids from field cases as a drench	Mar. 13 Repeated Mar. 29	Lambed	May 23	Normal		Not attempted
30	Vibrio culture 1 as drench; 0.5 cc. culture 1 intravenously	Mar. 13 Apr. 22	Lambed	May 25	Normal		Not attempted
38	Vibrio culture 1 as drench	Mar. 13	Lambed	June 15	Normal		Not attempted
199	Fetal fluids and vaginal discharges of ewe 207 as drench after Cu SO_4	Apr. 22	Lambed	May 22	Normal		Not attempted
200	Same as ewe 199	Apr. 22	Aborted	May 9	Small, weak dead in few hrs.; liver lesions	17 days	+
201	Vibrio culture 2 as drench after Cu SO_4	Apr. 22	Aborted	May 8	About 4 mo.	16 days	—
202	Vibrio culture 2 in conjunctival sac	Apr. 19	Lambed	May 20	Normal		Not attempted
205	5 cc. Vibrio culture 1 intravenously	Mar. 3	Aborted	May 14	About 3 mo.; congested liver	72 days	+
206	5 cc. Vibrio culture 1 intravenously	Mar. 30	Aborted	Apr. 9	About 4 mo.; dystokia	10 days	+
207	Vibrio culture 1 as drench after Cu SO_4	Mar. 30	Aborted	Apr. 20	About 4 mo.; liver lesions	20 days	+
208	Fetal fluids and vaginal discharge of ewe 206 after Cu SO_4	Apr. 9	Aborted	Apr. 27	Small, weak, dead in few hrs.	18 days	+

by oral administration alone was not given in their report.

Carpenter[4] injected two ewes intravenously with 10 cc. of pooled stomach contents of three aborted lambs containing the Vibrio. One aborted in 14 days, but the Vibrio could not be found. The *Streptococcus viridans* and a member of the colon-aerogenes group were isolated from the aborted lamb. The other ewe lambed normally.

Our experimental work upon pregnant ewes this spring has not been extensive, but the results have been interesting. Fifteen ewes were used in this work. Some were given the Vibrio in pure culture from the aborted lambs by the intravenous route. Others were given the pure culture as a drench without the use of copper sulfate. In some the pure culture was given as a drench immediately after the use of copper sulfate in an effort to get the culture to go directly to the abomasum. Some were given the fetal fluids and vaginal discharges orally both with and without the use of copper sulfate. In one pregnant ewe the pure culture was placed in the conjunctival sac. Table II summarizes the results of attempted infection.

In addition to the ewes listed in table II, two ewes given vaginal discharges and isolated cultures from field cases were not

pregnant, and two others given 5 cc. of culture 2 intravenously died as a result of the injection within 24 hours. It is shown that abortion was produced in six of the remaining eleven ewes, and one of the five ewes which did not abort received the culture only by the conjunctival route. Two which aborted received the culture intravenously in 5-cc. quantities. Four aborted which received the organism in the various materials as a drench after the use of copper sulfate.

DISCUSSION

It appears that the Vibrio was definitely established as the cause of abortion in three of the five outbreaks in Wyoming which were investigated. It is believed that the results obtained prove that the disease is transmitted from aborting ewes to noninfected ewes in from ten to 21 days or longer. It seems advisable, therefore, that when the veterinarian encounters abortion in ewes, he should recommend everything possible to prevent the disease from being transmitted to other ewes in the flock which might not be infected. This is especially true if the flock or band has a month or more of pregnancy remaining.

Special efforts were made in flock 4 to isolate a Vibrio, but without success. When the first aborted lambs from this flock proved negative bacteriologically, more aborted lambs were obtained. These were also negative for the Vibrio. Three organisms were isolated from these lambs. For lack of a better name, we called them a bipolar organism, an Actinomyces-like organism and a yeast-like organism. These were pooled and given intravenously and also as a drench to three pregnant ewes. No abortion or disturbances were produced and they were considered of no etiologic significance in the abortion in this flock.

The English workers reported finding a Vibrio in a flock with similar history and symptoms. They concluded that the Vibrio was the cause of the emaciated condition, numerous abortions and high death losses among the ewes in this flock. We feel certain that lack of feed was the cause of the trouble in flock 4. We believe vibrionic abortion was the cause of the trouble in flock 3, although bacteriologic findings were negative.

Inasmuch as the abortions in these five flocks occurred the past spring, it is impossible to say whether the disease will carry over to another year. English investigators[3] found 10 per cent sterility in ewes which aborted as a result of infection with the vibrionic type of the organism. Graham and Thorp[6] reported that the flock in which they found the Vibrio to be the cause had a reappearance of the disease the following year in the same ewes on the same premises. Baker and Stone[1] found that eight ewes which aborted from *Spirillum ovis* infection did not abort the following year. This is the first year we have encountered vibrionic abortion. The abortion reported upon previously was similar to that described by Howarth[9] and was caused by an organism of the Escherichia genera. This type of sporadic abortion has had no carry-over on either the premises or in the animals.

What the source of vibrionic infection was in these cases we are not prepared to state. In every case in which we isolated the organism, the ewes had access to stagnant, fecal-contaminated water. If there is a carry-over to the following season, it would be our opinion that a few of the ewes get infected from stagnant contaminated water. These in turn, when aborting, serve as a means of infecting others.

It is also our opinion that a continuance of abortion for six weeks to three months in a flock, when the animals have been moved and given unquestionable water and a change of feed, is often due largely to the fact that the animals become infected from ewes in the flock which have aborted.

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[9]Howarth, J. A.: *B. coli*-like organism causing abortion in sheep. Cornell Vet., xxii (1932), p. 253.

Methods of Evaluating the Efficacy of Anthelmintics

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The Food and Drug Administration of the Federal Security Agency and the Zoölogical Division of the Bureau of Animal Industry receive many requests for detailed information on methods to be employed in the critical evaluation of anthelmintics. Many of these inquiries have been made as the result of the activities of the Food and Drug Administration against misbranded worm remedies in the enforcement of the Food and Drugs Act of 1906, which has now been superseded by the Federal Food, Drug, and Cosmetic Act of June 25, 1938. The latter act not only places the responsibility for the efficacy claims for any anthelmintic squarely on the manufacturer or shipper, but in the case of a new drug, an appropriate application must be filed with the Administrator of the Federal Security Agency, accompanied by data of adequate scientific investigations to show that the drug is safe when used in the dosage, and with the frequency or duration prescribed, recommended or suggested in the labeling thereof. While the new drug section of the Food, Drug, and Cosmetic Act deals solely with the safety of the drug, the question frequently arises in the case of anthelmintics as to the justification of the anthelmintic claims made for the product on the label accompanying the application in which the claims are not based on adequate tests. While it is not possible to describe a test which will prove adequate under all conditions, a discussion of the various methods that have been employed in anthelmintic research may be helpful to those individuals who are seeking for the first time to determine whether a certain substance is or is not effective for the removal of helminths from domestic animals.

Tests Useful in Anthelmintic Research

In view of the fact that the various species of helminths may differ in habits and responses as widely as do the different domestic animals which they parasitize, the student of anthelmintics has to consider and to allow for all these variations as well as for the variable physical and chemical properties of the substances which may be used as vermicides. Consequently, all generalizations can not be made to apply to all specific cases, and in each instance the facts determined by carefully conducted tests must be taken into consideration.

A number of tests, which may be grouped under a few types, have been devised in the past to meet these variable conditions. The useful tests may be divided into two classes, namely, *in vitro* tests and *in vivo* tests. The latter class may be further subdivided into (1) the dilution egg-count test, (2) the critical test and (3) the controlled test. The kind of test to be employed must be determined by the purpose of the investigation, the means at the command of the investigator, and the parasite under consideration.

In Vitro Tests

In vitro methods which have been used in the past have varied greatly. Some investigators employ methods which consist of little more than a test tube in which the helminth is exposed to the chemical. Other investigators employ complicated apparatus, or they seek to interpret results by means of complex statistical analyses. Although special equipment or special analyses may be of value under some conditions, in general the more nearly the conditions of the *in vitro* test approach *in vivo* conditions, the more likely will the results obtained prove of value. Recently much publicity has attended the *in vitro* demonstration that pineapple juice would digest Ascaris. Probably tests conducted in the presence of digestive enzymes ordinarily present in the alimentary canal would have shown that this discovery was of academic interest only, since the enzymes contained

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in pineapple juice are inactivated by the usual digestive enzymes.* This example illustrates only one of the numerous obstacles confronting the investigator who attempts to apply the data obtained from *in vitro* tests to the conditions which prevail in the animal body.

The rapidity with which a large number of substances may be tested against helminths by the *in vitro* method is largely responsible for the popularity of this method, although the results obtained in this manner are not directly applicable to the conditions existing in the body of the host animal. If the investigator wishes to test a large number of substances, all of which belong to a homologous series of chemicals, or which exhibit some type of close chemical similarity, he may obtain *in vitro* results which are suggestive of results which may possibly be obtained *in vivo*, but even with substances having great chemical similarity, the investigator must be careful to make due allowance for many physical characteristics among which are the water solubility and the melting point of the chemical that is being tested.

In our experience closely related chemicals which differ in water solubility have in *in vivo* tests shown great variation in the anthelmintic efficacy, but comparatively little difference when tested *in vitro*. Conversely, variations in the melting point of related compounds, within the range of 35° to 100° C., have exerted great influence on the *in vitro* efficacy, but comparatively little on the *in vivo* efficacy. These are only two of the many factors which regularly produce a difference between the results of *in vitro* as compared with *in vivo* tests. As the combination of these factors varies with the characteristics of each chemical employed, with each host animal to be considered, and with each parasite involved, the nature and the direction of the variation can not be predicted with certainty for any particular chemical. Therefore, *in vitro* results can not be accepted as proof of anthelmintic efficacy or the lack of such efficacy until adequate *in vivo* tests have been performed.

*Since this was written, Kuitunen-Ekbaum (*Science*, xci, p. 241) reported experiments which demonstrate that pineapple juice is inactive *in vivo* in dogs.

IN VIVO TESTS

To determine with certainty the efficacy of any chemical as an anthelmintic, it is necessary to test it *in vivo* against one or more species of helminths. This process does not differ in fundamentals from the methods of scientific research employed generally in the biological and physical sciences. The subject matter of anthelmintic research is different from that of other fields, but the fundamental methods of all are identical; and these methods were described succinctly in a famous essay, "On the Educational Value of the Natural History Sciences," by Thomas H. Huxley, as follows:

> 1. *Observation* of facts—including under this head that *artificial observation* which is called experiment.
>
> 2. That process of tying up similar facts into bundles ticketed and read for use [in research, but not in practical therapeutics], which is called *Comparison* and *Classification*—the results of the process, the ticketed bundles, being named *General propositions*.
>
> 3. Deduction, which takes us from the general proposition to the facts again—teaches us, if I may so say, to anticipate from the ticket what is inside the bundle and finally—
>
> 4. Verification, which is the process of ascertaining whether, in point of fact, our anticipation is a correct one.

These are the fundamental methods to be used in testing anthelmintics, but it is especially dangerous in the field of therapeutic research to neglect the important step of verification. As Huxley points out, all natural philosophers for nearly two centuries following Harvey's great discovery believed that throughout the great phylum of the chordates, from Amphioxus to man, blood circulates in only one direction, but this simple deduction failed of verification in one peculiar group, the Ascidians, although it seemed to be based on the widest and safest inductions. Possibly von Hasselt's demonstration of the regular reversal of blood circulation in the Ascidians greatly disturbed the more dogmatic natural philosophers, but this misconception of nearly

two centuries' standing was responsible for no other important damage.

However, the therapeutist deals with drugs, which may be potentially harmful to the animal treated, either by direct toxic action of the drug or by failure of the drug to remove an injurious parasite for which it has been recommended. Obviously, the therapeutist can not afford to act upon an unverified deduction. For example, normal butyl chloride is effective for the removal of hookworms from dogs, and it is possible that the drug will prove effective against hookworms in man, but to prove the point it will be necessary to administer the drug experimentally to human beings.

In general, a drug which is very effective for the removal of one species of nematode will be somewhat effective for the removal of closely related worms from the same or closely related species of hosts; however, so many exceptions to this rule are known that no generalization is safe until adequate experimental data are available.

Dilution Egg Count.—In order to evaluate the effect of a drug against a parasite, a suitable method of testing must be adopted, since most helminths live in the body cavities where anthelmintic action can not be observed directly. The dilution egg count, first described in detail by Stoll and Hausheer, has been widely used for this purpose, and it is perhaps the best method for estimating the anthelmintic efficacy of drugs to be used in human medicine. When using this technic, the investigator places a measured quantity of feces in a flask and adds a measured quantity of fluid, usually a very dilute solution of sodium hydroxide. The flask is shaken until the fecal sample is thoroughly disintegrated, and a small measured quantity is withdrawn while the flask is being agitated sufficiently to maintain an even suspension of the eggs in the fluid. The egg content of the small sample is determined with the aid of a microscope, and in this way the number of eggs per gram of feces is calculated.

When applying this technic to anthelmintic research, the investigator makes one or more dilution egg counts before treatment and another, or several others, shortly after treatment has been administered. It is customary to assume that any changes between the two egg counts are due to the effect of the drug. To illustrate: If a stool eliminated by a man contains 3,000 hookworm eggs per gram of feces before treatment, and only 300 eggs per gram ten days after treatment, some investigators have assumed that the drug in question has removed 90 per cent of the female hookworms. One objection to this method is that in the case of a drug which depresses reproductive activity of the helminths without destroying the parasites, an indicated efficacy may result which is greater than the actual number of worms removed. Since other factors may cause wide variations in the egg production of helminths, data collected by the use of the dilution egg-count method alone can not be considered as proof of a high degree of efficacy, unless the investigator has devised reliable experiments for testing the systematic error which may cause a deviation from the true results. The dilution egg count can not be used in testing the effectiveness of a taeniacide, or of medicaments aimed at parasites whose eggs do not appear regularly in the feces.

Critical Test.—Since Hall advocated the use of the critical test, this method has been employed widely in anthelmintic research on domestic animals. In this test all feces eliminated by the experimental animal for three or more days following treatment are collected and examined carefully for worms; in this manner the number of worms removed by the treatment is determined. When the elimination of the worms has ceased, the animal is killed and the organs searched for helminths. The total number of worms eliminated in the feces plus the total found in the animal at necropsy is regarded as the total present at the time of treatment, and by simple arithmetical calculation it is possible to determine the percentage of efficacy of the drug. However, the critical test must be repeated on a sufficiently large number of animals before the results obtained can be regarded as reliable, since it occasionally happens that many of the helminths present

in the gastrointestinal tract of an animal may be eliminated for reasons that have nothing to do with anthelmintic medication. Furthermore, under experimental conditions almost any anthelmintic may exhibit, for no apparent reason, more or less wide variations in efficacy in different individual hosts of the same species. Repetition of the test, therefore, is the only certain way of determining the extent of this variation.

In the case of herbivorous animals, when given a slow-acting or partially effective anthelmintic, a certain percentage of the intestinal parasites removed are digested before they can be evacuated in the feces. This is the most serious source of systematic error in the use of the critical test. However, with the more effective anthelmintics now in use, this error is not important, because of the very high degree of efficacy exhibited by these drugs in spite of the fact that some parasites may be destroyed in the alimentary tract and are not found in the feces.

Critical tests may also be used to estimate the systematic error involved in the dilution egg-count method. Should the manufacturer rely entirely on dilution egg-count experiments to estimate the efficacy of a drug which he subsequently labels as a treatment for the removal of some specific worm, the manufacturer or shipper may find himself embarrassed if critical tests, conducted by the Food and Drug Administration in the enforcement of the Food, Drug, and Cosmetic Act, should demonstrate that the product will not accomplish what the label implies. For instance, certain foreign investigators have claimed, on the basis of fecal egg counts, that some organic arsenicals are effective for the removal of all strongyles from horses. Should the manufacturer conduct critical experiments on several horses, the results might show that the drug was very effective for the removal of small strongyles from horses, but almost wholly ineffective for the removal of the large strongyles. Since the common large strongyle, *Strongylus vulgaris*, is the most pathogenic nematode of the horse, it would be misleading to veterinarians, and to livestock men, to represent the product as effective against all species of equine strongyles. Instances somewhat similar to the above hypothetical case have been the subject of action in the enforcement of the Food and Drugs Act of 1906. Although the critical test is not the only method that might be used to evaluate data collected by the use of dilution egg count, it appears to be the most rapid, since the investigator can detect simultaneously errors due to several causes.

The critical test is not reliable for the determination of the efficacy of certain taeniacides unless adequate precautions are observed. Strobilae eliminated must not be counted as worms eliminated. As the scolex or head of a tapeworm is the growing point, it is the essential portion of the parasite and must be removed before it can be claimed that the worm has been eliminated or destroyed. Likewise, if the scolex is small, as is the case with most species, the host animal must be retained for two or three weeks so that the parasite may grow large enough to make its detection at necropsy certain. Occasionally the scolex or head of certain cestodes may be destroyed, presumably by digestion, after the drug has killed the parasite in the alimentary canal; in such cases critical tests are of little use.

Controlled Test.—The controlled test is the most time-consuming of the methods which may be employed to prove anthelmintic efficacy. This test has been found most valuable when artificial infestations are employed, because natural infestations, even among animals from the same source, vary so widely that a very large number of experiments must be conducted before significant results are obtained. With animals, like chickens, which may be raised easily under conditions which will prevent extraneous infestation with helminths, the method has proved useful, especially when investigating the value of drugs against those tapeworms which can not be studied satisfactorily by means of the usual critical test. In this type of test the experimental birds are infested by administering 50 cysticercoids of the tapeworm in question. About the time the tapeworms reach maturity one half of the fowls are treated and the other

half retained as controls. Two weeks after the treatment the fowls are killed and the number of tapeworms in all birds determined. The number of tapeworms recovered from the control birds is taken as the probable number of tapeworms present before treatment and the number found in the treated birds as the number not removed by the treatment. If ten treated and ten control birds are used in each experiment, consistent results can be obtained, but, of course, a single experiment is not sufficient to prove the therapeutic value of a taeniacide.

Summary and Conclusions

This brief discussion has dealt almost entirely with the methods which have been employed to evaluate the efficacy of anthelmintics used for the removal of helminths parasitic in the alimentary canal. While important parasites of man and domestic animals occur in other locations, the specific medication for the removal of such parasites has not been studied sufficiently to make possible any generalizations of interest on the subject. For special methods of research employed in these cases, interested investigators should consult the literature.

The safety of the drug is a matter of interest to all investigators, practitioners, livestock men and pharmacists, but it is outside the scope of the present article. Certainly no drug should be advocated as an anthelmintic unless some information on its therapeutic index is available.

The determination of anthelmintic efficacy involves the application of specialized knowledge to a special problem. No one of the methods outlined above is satisfactory for the investigation of all problems in this field. While combinations of one or more of these methods may be used in some cases, others may require the development of entirely new methods. From these considerations it should be obvious that anthelmintic research should not be attempted except by those who are familiar with the basic principles of helminthology and therapeutics. Even experienced helminthologists must proceed cautiously, and they must limit their conclusions to subjects which have been covered adequately by experimental data. Either the critical test or the controlled test, when properly carried out, will yield reliable information in many instances, but the other methods which have been used to study anthelmintic efficacy must be employed cautiously.

Vitamin A Deficiency in Horses*

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IN THE DEVELOPMENT of the vitamin A studies on various species of farm animals at this experiment station it was observed that rations fed to deplete the experimental animals were not greatly different from what would be considered normal rations for horses. Where yellow corn is not available, practically all concentrates fed to horses are low in this factor. When the roughage consists of grain hay or even poor quality timothy hay, the intake of this vitamin with such forage may not be sufficient for minimum requirements. Such a possible deficiency would be alleviated by the widespread practice of turning horses out to graze on green pasture for varying periods of time during the summer season. It frequently happens in city horses and particularly in army horses stationed in semiarid regions that opportunity for pasturing does not exist.

Abnormalities of nutritional origin in army horses have been reported from various parts of the world. Klemola[1] presented data on marked alterations in the hoofs of horses in the army of Finland. In that country the army-horse rations consisted of oats, dry hay and oat straw; there was no opportunity for pasture and rarely any other green fodder. Much of the hay becomes bleached and leached in curing and samples submitted to Scheunert and Schieblich[2] in Leipsig showed no vitamin A to be present in it. Ten to 15 per cent of the cavalry horses and 20 to 30 per cent of the artillery horses, constituting the older animals, in the spring of the year particularly showed marked horn changes in the hoofs. This included brittleness, abnormal horn growth with furrows and cracks, making shoeing difficult. A spongy crust was often present on the surface of the hoof wall toward the heels and the surface throughout was rough and scaly. The condition was markedly alleviated in experimental groups of horses totaling 113 head to which green pasture, A.I.V.* silage or cod liver oil was added to the basal ration, while no improvement occurred in others the basal ration of which was supplemented with potatoes or vitamin D: (vigantol). Careful histologic studies of the hoof changes showed a hyperkeratosis or overgrowth of the perioplic layer representing the stratum corneum of the skin epidermis and a marked atrophy of the tubular layer secreted by the coronary band.

General debility of army horses due to a restricted diet, such as reported by Chatelain[4] in French army horses in French Indo China, is reported as common to those with experience in the East by Edwards[5] of the British army in his elaborate discussion of carotene deficiency and some common unsoundnesses in horses. Chatelain improved this condition by injecting cabbage pulp, cod liver oil and egg yolk into the affected animals.

The first definite symptom of vitamin A deficiency in animals including the horse is night blindness. This condition was reported by Meadows[6] in horses and camels in Persia without knowledge as to its cause. It was stated that in military operations night blindness becomes serious, as affected animals are extremely nervous, dangerous and useless for operations in the dark.

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*From the Animal Husbandry Division, University of California; the investigational work reported herein was financially supported and became coöperative, under a Bankhead Jones Project, with the U. S. Bureau of Animal Industry.

[1]Klemola, V.: Biedermanns Zbl., B. Tierernähr., v (1933), p. 657.

[2]Scheunert, A., and Schieblich, M.: Biedermanns Zbl., B. Tierernähr., vi (1934), p. 133.

*Silage specially prepared by the use of hydrochloric acid after the method outlined by A. I. Virtanen[3] to preserve its vitamin A content.

[3]Virtanen, A. I.: Cattle Fodder and Human Nutrition (Cambridge University Press, 1938), pp. 1-108.

[4]Chatelain, P.: Bul. Acad. Vét. France, vi (1933), p. 452.

[5]Edwards, J. R.: J. Roy. Army Vet. Corps, ix (1937), p. 3.

[6]Meadows, D.: Vet. J., xxvi (1919), p. 140.

As early as 1904, the idea of a single systemic disturbance rather than trauma entering into a causal relation to the abnormalities of bones and joints in horses was suggested by Vivien and Augustin.[7] They considered all arthritis in these animals the same, differing only in secondary points. First would appear simple ecchymoses or slight cartilaginous desquamation, then ulceration with cartilage disappearing at the borders. Granulating osteophytes develop at the margins and may unite one bone to the other, resulting in ankylosis and even exostosis. They suggest a succession occurs of osteitis, arthritis, ankylosis and exostosis. Thus spavin, for example, long considered a morbid entity, would be only a symptom, rather than a syndrome.

The following year Williams, Fisher and Udall[8] wrote on the spavin group of lamenesses and included a variety of conditions affecting other joints. They called attention to the geographic distribution of these abnormalities and presented evidence of their being almost enzoötic in one area and practically unknown in another. They agreed with the idea advanced by Vivien and Augustin[7] that these abnormalities constituted a systemic disturbance and laid stress on nutrition as playing the essential rôle in their etiology.

Hare[9] studied a disease of joints of horses in England tending to chronicity which he considered was probably specific but due to no known specific cause and for which he used the term chronic arthritis. He examined *ante mortem* and *post mortem* a total of 146 horses, many of which were killed for meat purposes, and found 21, or 14 per cent, affected with chronic arthritis and four lame from other causes. The animals varied in age from 6 to 28 years and their owners had no precise recollection of the initial signs of the trouble. Bacteria could not be incriminated as the causal agent. The cartilage changes consisted of congestion followed by a dull opaque appearance and uneven surface, the so-called fibrillation. The second stage consisted of erosion duplicated upon opposing articular surfaces. This was followed by furrowing or ridges. There was ankylosis in affected hock joints and eburnation of exposed bone. He stated it was reasonable to consider the proliferative changes in the marginal zone as analogous to those on the nonarticular surface of the bone which resulted in exostosis.

Mitchell[10-14] has presented an elaborate hypothesis to the effect that many of the unsoundnesses of horses, including such widely different conditions as cataract, roaring, shivering, stringhalt, navicular disease and the bony exostoses, ringbone, side bone, and spavin, listed under the horse-breeding acts of the British Isles and the stallion-registration laws of the United States, are not hereditary. On the other hand, he believes them to be of dietary origin, involving mineral imbalance, particularly calcium, and phosphorus as well as vitamin deficiency, including possibly A, C and D, with stress being laid on A. He has classed the whole group provisionally under the term rheumatic disease. A great amount of clinical observations and some careful and complete postmortem examinations, but little controlled experimental data, have been assembled to support the general thesis. Nevertheless, time, development of knowledge and experimental data are tending to produce evidence that the intriguing and stimulating Mitchell hypothesis, although probably too broad in its generalities, is obtaining partial support.

Kelser and Callender[15] reported upon the high incidence of what they termed equine degenerative arthritis in U. S. Army animals in the Panama Canal Zone. In the spring of 1935, a survey divided the animals into three groups. In the first group, comprising 48 per cent of the horses and

[7] Vivien and Augustin: Rev. Gén. de Leclainche. (Reviewed in Amer. Vet. Rev., 1904, pp. 712-715).

[8] Williams, W. L., Fisher, C. W., and Udall, D. H.: Proc. A. V. M. A. (1905), pp. 283-348.

[9] Hare, T.: Vet. Rec., xxxix (1927), pp. 411-421, 433-440.

[10] Mitchell, W. M.: Vet. Rec., xlii (1930), pp. 89, 535.

[11] *Ibid.*: Vet. Rec., xliii (1931), pp. 412, 1126.

[12] *Ibid.*: Vet Rec., xlv (1933), p. 918.

[13] *Ibid.*: Vet. Rec., xlvii (1935), p. 1501.

[14] *Ibid.*: Vet. Rec., xlviii (1936), p. 1365

[15] Kelser, R. A., and Callender, G. R.: Vet. Med., xxxiii (1938), p. 307.

34 per cent of the mules, a total of 352 animals were so badly affected that they were recommended for immediate disposal. The second group, recommended for retention for one year, comprised 249 animals, being 32 per cent of the horses and 24 per cent of the mules, while only 20 per cent of the horses and 42 per cent of the mules, or 346 head, were in condition to be recommended for indefinite retention. This affection was believed to be of dietetic origin and the standard ration of 14 lb. of grain hay and 8 lb. of oats was changed in 1935 so that 5 lb. of alfalfa hay replaced an equal amount of the grain hay, the effect of which has not been reported.

Postmortem examinations were made on 54 head and all of them had abnormalities in the articular cartilages which were the outstanding and characteristic lesions found. In a number of cases the joint lesions were so extensive as to be out of proportion to the degree of lameness which had been shown. They did not find any evidence that the condition was osteomalacia and they had cases of the latter in mules from a nearby banana plantation to use for comparison. The authors stated: "In many cases with marked erosions of the articular cartilages of the leg joints we have found the hard bone structure to be normal. They were dense in texture, flintlike in hardness and showed nothing which by any possible stretch of the imagination could be taken as evidence of osteomalacia." In their cases no evidence of a relationship was found between the degenerative joint changes and the productive alterations arising in the periosteum of the body of the bone producing bone spavin and ringbone. On the other hand, they reported the army veterinarians had observed high incidence of spavin, ringbone, etc., in army animals in Panama and that improvement followed the addition of alfalfa hay to the diet.

In the face of these conditions it was thought to be desirable and means were provided to include horses in the studies of vitamin A deficiency of farm animals at this station. Data on minimum requirements of vitamin A in these horses have already been published by Guilbert, Howell and Hart.[16] The data in this paper deal particularly with the combined vitamin A deficiency and varying calcium and phosphorus ratios with particular reference to the extensive joint lesions present in all of the cases.

Facilities, Animals and Rations

The experimental animals, eleven in number, were all purebred Percherons born and reared in the University Farm Stud. Their history as well as that of their parents was known. Prior to being placed on experiment these animals received normal rations including alfalfa hay and irrigated pasture.

All of the animals were kept throughout the experiment in four adjoining corrals partially roofed. The ground area not roofed was covered with cement while a dirt floor was under the shed portion. Water was supplied in cement drinking troughs. A cement runway extended along the entire length of the four corrals at the opposite end from the shed, and in this runway the animals were tested for night blindness.

The corrals were well arranged to prevent the animals from getting any food except that supplied to them in the mangers under the shed.

Throughout the experiment the concentrate rations contained barley, oats, wheat bran and linseed meal. To this was later added beet pulp and cottonseed meal. They were fed to the first series at a daily average intake of 12 to 13 lb., and in the second series without straw this was increased to from 17 to 19 lb.

The roughage when used consisted of barley straw. As the experiments progressed, the horses depleted slowly and analyses made of the straw showed it to contain .05 to .10 mg. of carotene per 100 Gm. It was, therefore, withdrawn on June 6, 1938, after horse 1 and filly 4 died.

All of the concentrates were low in or devoid of vitamin A, but analyses made of the concentrate mixture as fed showed .01 to .02 mg. of carotene per 100 Gm. of the feed. The rations are shown in table I.

On these rations including the straw the animals developed a marked tendency to chew on the woodwork of the fences, mangers and posts. In order to partially prevent this the woodwork was covered with woven wire, barbed wire or metal strips.

Experimental Procedure

First Series of Horses

These animals were started on experiment February 8, 1936. They consisted of four

[16] Guilbert, H. R., Howell, C. E., and Hart, G. H.: J. Nutri., xix (1940), pp. 91-103.

TABLE I—Age, Weight, Date of Starting, and Death of Experimental Horses with Rations and the Ca:P Ratio, Appearance and Days Required to Produce Night Blindness

Animal Group Number		Date Started	Age in Days	Weight in Pounds	Date of Death	Days on Exp.	Ration Number	Total Ca. % in 100 Lb.	Total P % in 100 Lb.	Ratio Ca:P	Date Night Blindness Appeared	Days Required
Group A	Horse 1	2/8/36	370	1,105	11/23/37	652	I	0.08	0.40	1:5	6/24/37	501
	Filly 2	2/8/36	284	820	9/16/38	950	I IA*	0.08 0.11	0.40 0.70	1:5 1:7	6/15/37	492
Group B	Horse 3	2/8/36	376	930	3/23/39	1,139	II IIA*	0.42 0.70	0.39 0.69	1:1 1:1	6/24/37	501
	Filly 4	2/8/36	324	965	3/8/38	758	II	0.42	0.39	1:1	10/28/37	627
Group C	Horse 5	5/25/38	444	1,170	9/6/39	469	III	0.10	0.71	1:7	7/6/39	407
	Horse 6	5/25/38	410	1,240	3/7/39	286	III	0.10	0.71	1:7	———	
Group D	Filly 7	5/25/38	440	1,035	3/30/40	675	IV	0.44	0.39	1:1	8/8/39	440
	Horse 8	5/25/38	383	1,075	3/17/40	662	IV	0.44	0.39	1:1	1/16/39	236
Group E	Horse 9	5/25/38	420	1,120	7/22/38	58	V	1.04	0.39	2:1	———	
	Filly 10	5/25/38	441	1,145	10/3/39	496	V	1.04	0.39	2:1	3/16/39	295
	Horse 11	9/16/38	119	600	6/10/40	633	V	1.04	0.39	2:1	7/21/39	308

RATIONS

Number I		Number II		Number III		Number IV		Number V	
Barley	30.0%	Barley	30.0%	Barley	30.0%	Beet pulp	55.0%	Beet pulp	53.5%
Oats	30.0	Oats	30.0	Oats	30.0	Barley	10.0	Barley	10.0
Wheat bran	30.0	Wheat bran	30.0	Wheat bran	30.0	Oats	10.0	Oats	10.0
Linseed meal	10.0	Linseed meal	8.5	Linseed meal	5.0	Wheat bran	10.0	Wheat bran	10.0
Straw daily	10.0 lbs.	$CaCo_3$	1.5	Cottonseed meal	5.0	Linseed meal	10.0	Linseed meal	10.0
		Straw daily	10.0 lbs.			Cottonseed meal	5.0	Cottonseed meal	5.0
								$CaCo_3$	1.5

Number IA	Number IIA
Same as I without straw*	Same as II without straw*

*No straw in any of the diets after June 6, 1938.

head varying in age from 284 to 376 days, and weighing from 820 to 1,105 lb. They were divided into two groups.

Group A, consisting of horse 1 and filly 2, received ration I and I A (see table I). This ration had a Ca-P ratio of 1 to 5.

Group B, consisting of horse 3 and filly 4, received the same diet except that 1½ lb. of $Ca\ CO_3$ replaced an equal amount of linseed meal, thus bringing the Ca-P ratio to the level of 1 to 1.

The animals received straw as a roughage at the rate of about 10 lb. per day. After being on the diet for periods varying from 492 to 627 days, all of the animals showed night blindness.

Group A, Horse 1.—This animal was night blind on June 24, 1937, 501 days after going on the experimental diet. He received several minimum vitamin A treatments in the form of alfalfa meal or cod liver oil. This animal showed swelling and lameness in the leg joints with stocking below the knees and hocks which was sufficiently serious to interfere with proper testing for night blindness. The joint affection became rapidly worse after the withdrawal of vitamin A. Cod liver oil, 6 cc. per day, was administered from November 4 to 8, and an average of 35 cc. per day from November 9 to 23. The animal went down November 8 and was not able to rise until November 14. He developed decubital sores, marked weakness and anorexia and was killed *in extremis* on November 23, 1937.

Postmortem examination showed marked

TABLE II—Analyses of Rib Bone from Each of Two Horses in First Series

	Ash	Ca	P	$CaCO_3$	$Ca_3(PO_4)_2$	$\frac{Ca_3(PO_4)_2}{CaCO_3}$
Horse 1	57.40	21.59	9.78	7.59	47.60	6.3
Filly 4	62.13	24.18	10.52	8.78	51.30	5.8

joint lesions with excess synovial fluid in all the major joints of the fore and hind legs. These lesions involved the articular cartilages and underlying bone with a rarefying inflammation resulting in irregular ulcerated surfaces of varying size in the cartilages and some pitting of the underlying bone. Figure 1 shows the bones of this animal after the soft tissues had been removed by simmering in ammoniated water at a temperature of about 85° C. Notice the marked pitting of the head of the femur beneath the articular surface. The joint lesions were similar to those described and illustrated by Kelser and Callender. In addition there was rarefaction of the bone away from the articular surfaces, also shown in figure 1. (See tables II and III.) Evidence of this condition also was manifested in other bones, including the phalanges, by marked pitting of the surface. Figure 2 shows a photomicrograph of the cannon bone of this animal compared with that of filly 4.

The molar teeth in this case were worn down close to the gums. This being the first animal coming to postmortem examination, it was thought at the time that the rather wide Ca-P ratio might be largely responsible for the condition of the bones and the joint lesions. The cod liver oil therapy during the last 19 days of life resulted in the liver tissue showing 112 blue units of vitamin A per gram.

Group A, Filly 2.—This animal became night blind on June 15, 1937, 492 days after going on the experiment. She was given several periods of vitamin A treatment with alfalfa meal or cod liver oil in the study of minimum requirements. Straw was removed from the ration on June 6, 1938, and thereafter none was fed to any of the animals. She showed marked lameness and had several periods of incoördination and actual convulsions. This was followed by difficulty in rising in the morning. Finally, for several days the animal was unable to rise and was killed on September 16, 1938.

Postmortem examination showed joint lesions as in the previous case. The bones were of normal density in marked contrast to the other animal in this group. (See table II.) The internal organs were normal and the liver was negative for vitamin A.

Group B, Horse 3.—This animal was found to be night blind on June 24, 1937, 501 days after going on the experiment. He was given several periods of vitamin A treatment in the study of minimum requirements. On May 4, 1938, although night blind, he bred filly 2 three times and his seminal fluid contained active sperm. This horse was in the same corral with filly 2 after horse 1 and filly 4 died.

The eyes of this and other animals were examined August 23, 1938, with an ophthalmoscope by C. K. Mills, ophthalmologist at the Woodland Clinic. Both corneas were sufficiently clouded at the time to prevent clear observation of the fundus and disc, but still not as clouded as those of filly 2. No evidence of swelling or elevation of the disc could be detected in comparing the night-blind with normal animals.

During the periods of night blindness this animal made a snoring noise in breathing, even when standing quietly. This improved on vitamin A therapy. Straw was removed from the ration on June 6, 1938. Following this, breathing became noisy and labored. A rather severe nasal hemorrhage occurred on February 15, 1939, and the animal went down but regained his feet. A second similar attack proved fatal on March 23, 1939.

Postmortem examination showed no respiratory alterations to account for the great difficulty in breathing. This was evidence that muscular paralysis from nerve involvement

TABLE III—Density of Femur, Astragalus and Short Pastern Bones from Experimental Horses Expressed in Grams per Cubic Centimeter*

Horse Number	Femur	Astragalus	Short Pastern
Horse 1	0.39	0.75	1.00
Filly 2	1.00	1.19	1.20
Horse 3	0.71	1.05	1.00
Filly 4	0.76	1.29	1.25
Horse 5	0.78	1.20	1.20
Horse 6	0.66	1.10	.96
Filly 7	1.02	1.12	1.27
Horse 8	0.89	1.16	——
Horse 9	0.80	1.10	1.20
Filly 10	0.82	1.37	1.24
Horse 11	0.74	1.21	1.27
Normal*	0.87	1.32	1.30

*Normal was 7 years old when killed. Did not have a normal the same age as the experimental horses.

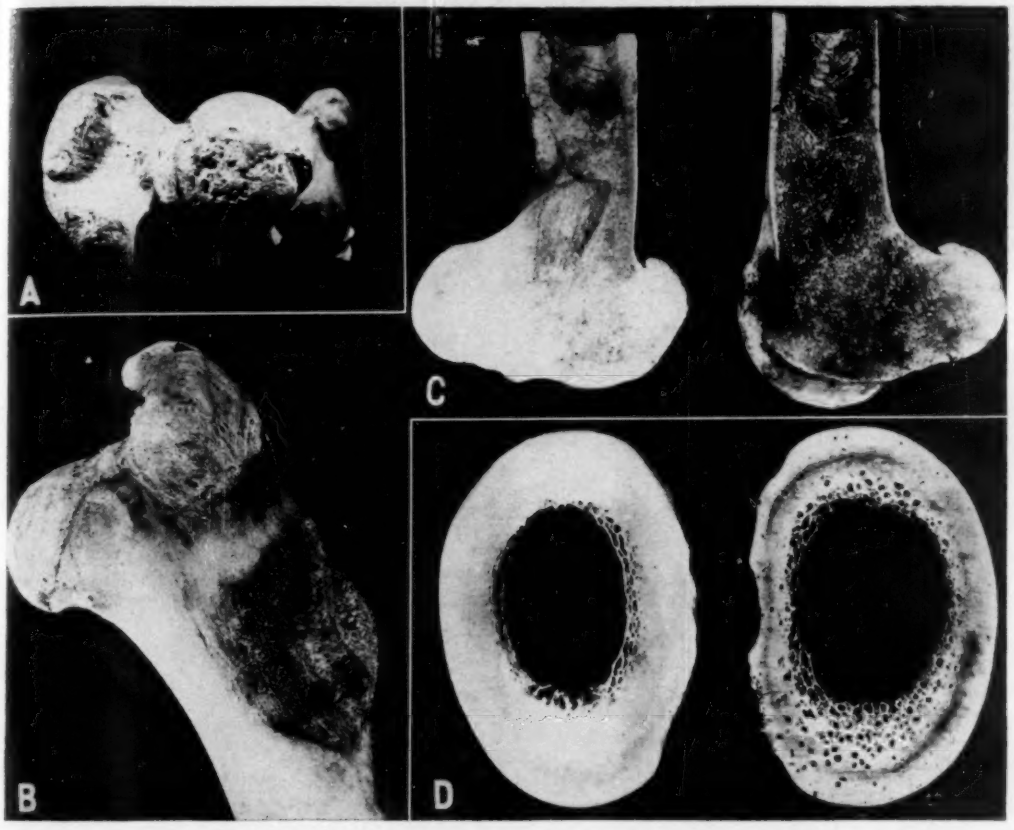

Fig. 1. Changes in bones of horse 1. (A) Head of femur with marked pitting beneath articular cartilage. (B) Rarefaction of shaft of femur about area of muscular attachment. (C) Sagittal section of distal end of femur compared with normal bone of filly 4 (left). (D) Cross section of cannon bone compared with normal bone of filly 4 (left).

supplying the respiratory muscles might have been the cause. It could not be demonstrated at the postmortem examination. The arytenoid cartilages were normal and the clinical condition did not resemble roaring. There was much greater effort required for the animal to inspire than to expire air. Marked joint lesions were present similar to those already described. (See figure 3.)

The animal had been on the ration for 1,139 days and died at 1,515 days of age.

Group B, Filly 4.—This animal became night blind on October 28, 1937, 627 days after going on experiment. She recovered on minimum vitamin A intake in the form of alfalfa meal in 32 days. Vitamin A therapy ceased December 1, 1937, and she was again night blind January 24, 1938. This filly was bred on February 17, 1937, by horse 3, group B, kept in the same corral. Further breeding occurred at subsequent heat periods. A positive rat test for pregnancy was obtained on June 15, 1937. This animal died on March 8, 1938, from general peritonitis accompanying foaling. The parturition required manual assistance because of malpresentation. The foal was fully developed, weighed 113 lb. and may have been alive when labor started, but was dead when delivered. Its liver was negative for vitamin A. The dam had been night blind 131 days before labor occurred, but had been given one minimum treatment with alfalfa meal, and straw was in the ration. The hoofs of this mare showed the most marked changes in the periople from vitamin A deficiency of any of our cases. (See figure 4.)

Postmortem examination demonstrated the same joint lesions, particularly in both shoulder joints, as shown in the other three animals, although this animal and horse 3 had constantly been on a ration having a Ca-P ratio of 1 to 1. Figure 5 shows the weight curves of the experimental horses.

SECOND SERIES OF HORSES

On May 25, 1938, six more horses ranging in age from 410 to 444 days were di-

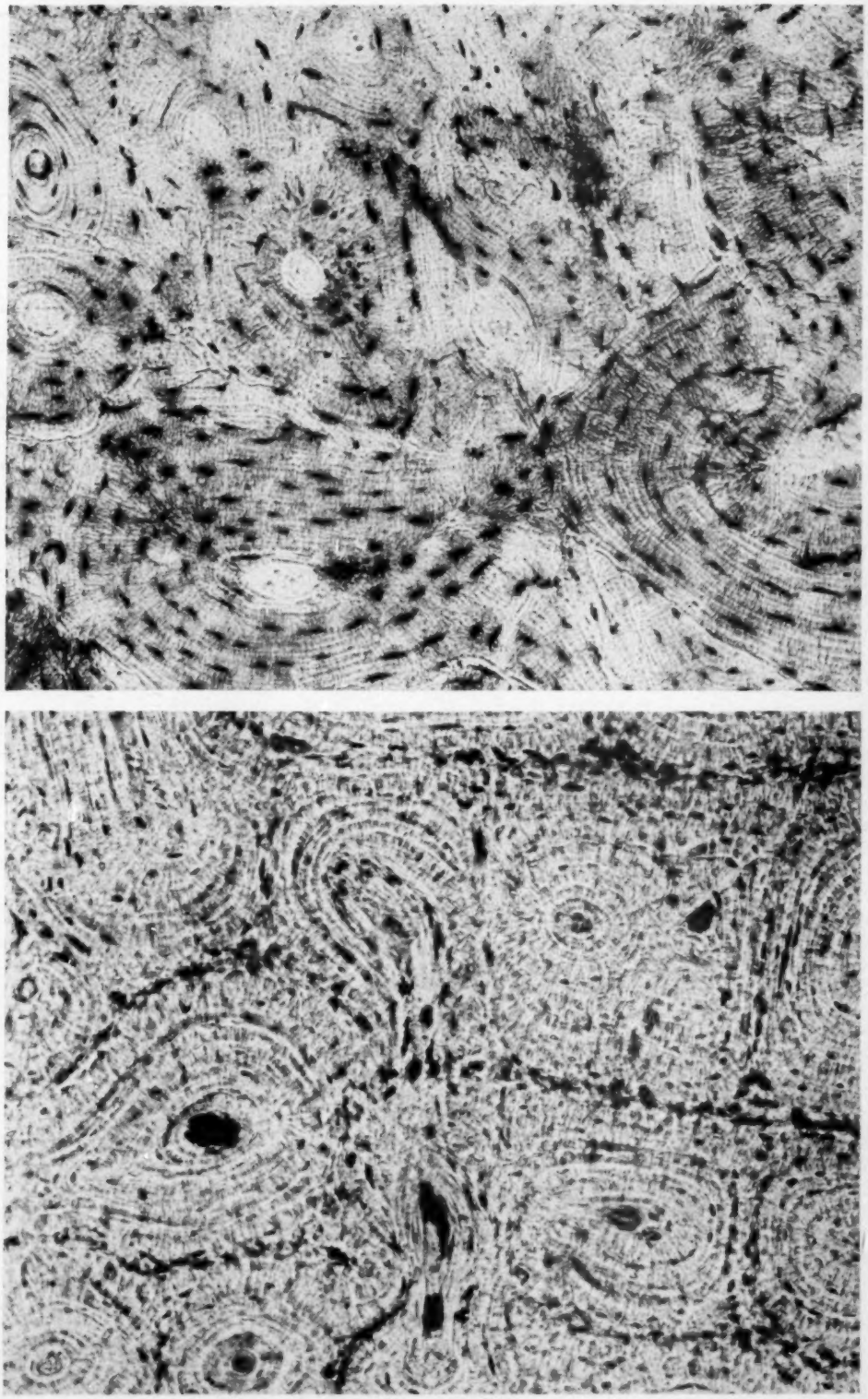

Fig. 2. Sections of the cannon bones of filly 4 (upper) and horse 1 (lower). Sections ground with emery, unstained (x 170).

vided into three groups and placed on the deficient concentrate rations. (See table I.) No straw was supplied. The reduced vitamin A intake from the exclusion of straw caused night blindness to develop earlier in these animals than in the case of the first series of horses.

They were divided into three groups as follows: Group C, horses 5 and 6, were placed on ration III with a Ca-P ratio of 1 to 7. The concentrates were the same as for the first series group A animals except that 5 lb. of cottonseed meal replaced an equal amount of linseed meal.

Group D, filly 7, and horse 8 were given ration IV, which had a Ca-P ratio of 1 to 1.

Group E, horse 9 and filly 10 received ration V, which was the same mixture as group D received except that 1½ parts of Ca CO_3 replaced an equal amount of beet pulp, thereby making the Ca-P ratio of this ration 2 to 1. The beet pulp was placed in the ration to provide bulk in the absence of roughage and to narrow the Ca-P ratio.

Group C, Horse 5.—This animal became night blind on July 6, 1939, 407 days after going on experiment, and was given minimum vitamin A therapy. In December 1938, he showed lameness in the left front leg from what was probably an injury seven months after going on the experiment. Complete recovery occurred and no other lameness was manifested.

Death occurred suddenly in this case on September 6, 1939, when the horse was still in reasonably good condition. The animal was down 24 hours before death and showed some evidence of encephalomyelitis, other cases being in the general vicinity at the time. The Division of Veterinary Science of the University of California found no toxin in the brain and histologic sections failed to show the polymorphonuclear cell infiltration about the blood vessels and in the brain tissue, which excluded this infection.

Postmortem examination showed the same joint lesions in the major leg-joint cartilages. While they were not as marked as in the other cases, they confirm the statement of Kelser and Callender that the lesions in the joints were out of proportion to the amount of lameness shown by the animal during life. The lack of more extensive joint lesions in this case was accounted for by the rather sudden death while still in good physical condition. The internal organs were normal and, in the absence of encephalomyelitis, central nervous system involvement in the vitamin A deficiency might explain the early fatal termination. The liver tissue was negative for vitamin A.

Group C, Horse 6.—Night blindness was never definitely demonstrated in this animal. This may be accounted for by the fact that he became so lame that a proper test could not be obtained. Carotene treatment was given during the last 48 days of life and 250 blue units of vitamin A per gram of liver tissue were found *post mortem.* (See "Discussion.") This animal grew rapidly and at death had a height at withers of 67 in. while the other animals measured 3 to 4 in. less.

He became lame with incoördination and had difficulty getting down and up and in moving about the corral, finally being in such extreme condition that he was killed March 7, 1939.

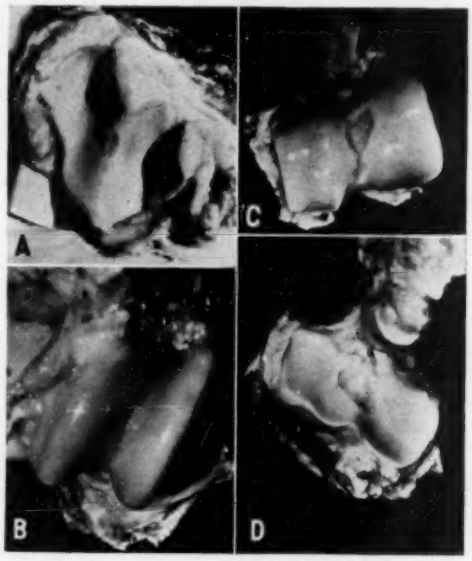

Fig. 3. Joint lesions in horses 3 and 11. (A) Ulceration on central ridge of articular surface of distal end of tibia, horse 3. (B) Similar condition on opposing surface of astragalus. (C) Ulceration on articular cartilage of distal end of humerus, horse 11. (D) Similar condition on opposing surface of radius.

Postmortem examination showed widespread and well-advanced joint lesions similar to those already described. The knee joints were swollen. The articular surfaces of the cartilages of these joints were not involved except for slight roughening at the margins.

Group D, Filly 7.—This animal was started on experiment May 25, 1938, and shortly became pregnant to horse 8 kept in the same corral. She foaled normally April 23, 1939, but the foal was unable to nurse. After three days of effort on the part of attendants including the administration with difficulty of small amounts of milk from the mother and about 750 cc. of cow's milk, the animal still would

not suckle and it was destroyed. A vitamin A determination on its liver tissue gave negative results. Failure to nurse in young animals is a symptom of vitamin A deficiency. The joints of the foal were normal. On April 26, 1939, the dam was partially defective in vision on a night-blindness test. She was again bred by horse 8 when he was night blind on May 10, 1939, and became pregnant. The animal was night blind on August 8, 1939, 440 days after going on the experiment. She was given one vitamin A treatment with cod liver oil to cure the night blindness.

On October 13, 1939, 400 mg. of ascorbic acid

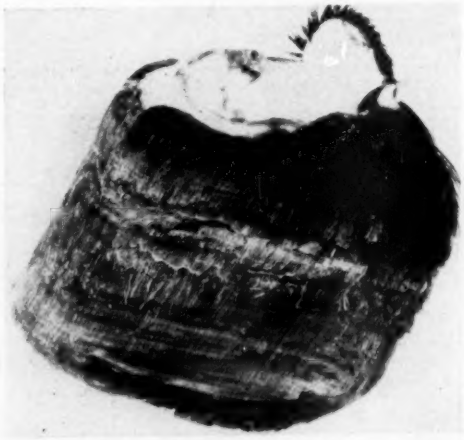

Fig. 4. Hoof of filly 4, group B, showing marked scaling of the periople.

was added daily to the ration in order to eliminate possible vitamin C deficiency. The animal developed soft, puffy swellings about the hocks. Beginning January 19, 1940, 4 oz. of brewers' yeast of known biologic value was added to the basal ration to insure adequate vitamin B intake. This resulted in some temporary improvement in appetite, appearance of the coat and reduction in size of the swellings around the hock joints.

After several weeks the coat again became roughened, hock swellings increased, and there developed marked lacrymation and dyspnea on slight exercise. The animal went down from attempts of horse 11 to ride her and died March 30, 1940. The dyspnea was similar in all the cases except for the hemorrhages shown only by horse 3. There was difficulty on inspiration so severe that the nostrils were dilated, mouth open and tongue extended with excessive flank movements in diaphragm contraction. The animal would break out in excessive sweating, go down and lie prone, and death would appear to be imminent. If death did not occur from asphyxia, the relaxation resulting from exhaustion would be followed by improved breathing and gradual recovery. The condition is identical with the syndrome of paraplegia from lead poisoning where there is paralysis of the muscles of both arytenoid cartilages.

Postmortem examination showed the same typical joint lesions despite the addition of vitamins B and C in the diet. There was a fully developed foal in the uterus conceived on the May 10, 1939, service previously mentioned. The liver of both mare and foal were negative for vitamin A. The joints of the foal were normal.

Group D, Horse 8.—This animal was started on experiment May 25, 1938, at 383 days of age. He became night blind January 16, 1939, after 236 days on the experiment. Several vitamin A treatments with cod liver oil were given in the study of minimum requirements.

On October 13, 1939, when the animal was again night blind, 400 mg. daily of ascorbic acid was added to the diet. No improvement was observed from this addition. Symptoms progressed including weakness, lacrymation, mottling of the cornea, swelling of the hock joints, poor appetite, difficult breathing on slight exertion and lying down a great deal. Finally he was found down with marked dyspnea on March 17, 1940, and death occurred that day. Postmortem examination showed the characteristic joint lesions. The internal organs and respiratory passages were normal. The liver was negative for vitamin A.

Group E, Horse 9.—This animal died July 22, 1938, from encephalomyelitis, 58 days after going on the experiment. He was replaced by horse 11, to be discussed later.

Group E, Filly 10.—This animal showed night blindness on March 16, 1939, 295 days after the experiment was started. Lameness also developed early and became very severe. At times this animal showed an exaggerated and atypical form of stringhalt in both hind legs (fig. 6). The leg would be flexed excessively on attempting to walk, thus raising the hoof almost to the abdomen in the worse stages. Excessive flexion continued for varying periods before again extending the leg and returning the foot to the ground. This might have been due to pain in the joint, but the other animals did not show it. At times incoördination was evidenced in the hind quarters, with a tendency to lean against objects to keep from falling. Muscular shivering was present at these times, probably due to general weakness, but the animal in some respects might have been considered a shiverer. There finally developed difficulty in rising over the last several weeks of life. Upon gaining her feet, marked shivering would occur for several minutes.

An English groom at the University Farm who had seen the syndrome known as shivers in England was of the opinion that this animal would be considered as a case of this trouble.

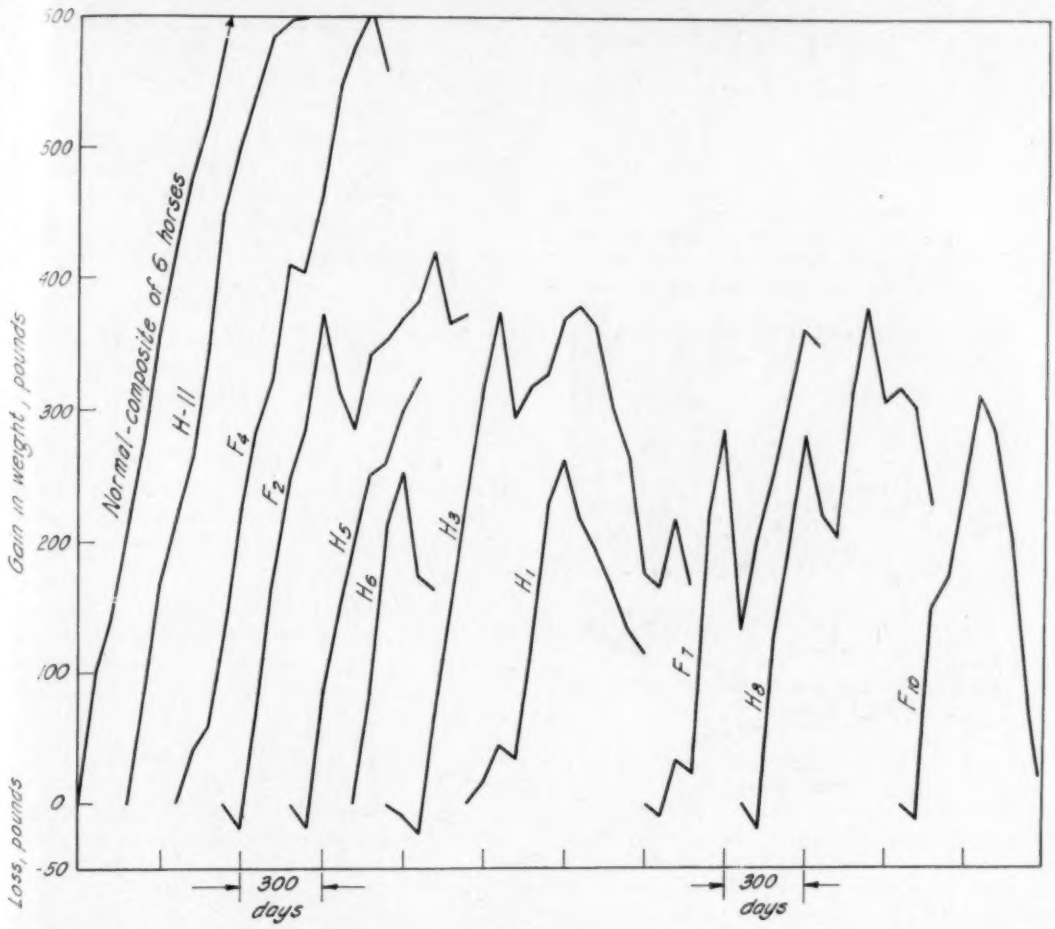

Fig. 5. Weight curves of ten of the experimental horses from time they were started on experiment until death. Normal growth curve at left.

In her family there was another animal in the stud but not on experiment which also showed some evidence of being a shiverer, but the symptoms were very mild compared with those shown by this experimental animal. This animal did not manifest marked respiratory difficulty but showed great weight loss (fig. 5) and severe weakness resulted therefrom. She did not become pregnant although in the same corral with a male. Weakness became more marked, appetite failed, and the animal died on October 3, 1939.

Postmortem examination showed extensive and severe joint lesions. The internal organs were normal and vitamin A determination on the liver tissue was negative.

Group E, Horse 11.—When horse 9, group E, died, it was desired to replace it. A 2-month-old foal born May 20, 1938, was the only animal available. It was left with its mother on alfalfa pasture during the summer and placed on experiment September 16, 1938, at 119 days of age. The diet was the same as the horse which it replaced had received. (See table I.)

On this regimen it developed definite night blindnes after 308 days, on July 21, 1939. One minimum curative treatment with cod liver oil was given. Night blindness again developed September 14, 1939. On October 13, 1939, 400 mg. of ascorbic acid was added to the daily diet and on December 14, 1939, 4 oz. of brewers' yeast also was given daily.

As in filly 7, temporary mild improvement followed the addition of the yeast. It was of short duration and the animal's coat became roughened, with poor appetite, weak stumbling gait, sweating, and labored breathing on slight exertion.

On June 10, 1940, at 11:30 a. m., the animal was standing under the shed as usual. He had not eaten all of the morning feed. Five

pebbles were thrown over the manger one after the other to cause the animal to move about. As each pebble hit him, it caused great excitement and efforts to move revealed weakness and incoördination. In moving about 20 ft., he passed from under the shade of the shed. The glare of the sun or the heat of the very warm day disturbed the animal and with great effort he turned around. Respiration became affected and in staggering forward there were three or four whistling inspirations as a hemiplegia. The animal went down as he reached

Fig. 6. Filly 10, group E, with atypical stringhalt.

the manger with profuse sweating, lay prone, nostrils dilated, mouth open and tongue protruding with marked flank movements. Death appeared imminent. After about 15 minutes he rose to his sternum and was left in this way at 12 o'clock. At 12:45 the animal was found dead.

Postmortem examination showed the internal organs to be normal. The liver was negative when tested for vitamin A. The leg joints showed the typical lesions of the previous cases, but the changes in the joint cartilages were as severe as in any animal in the experiment, showing that the daily administration of amply sufficient amounts of vitamins B and C for five and eight months, respectively, had had no effect in alleviating the condition. (See figure 3.)

Blood Examination

During the long period that these horses were on the restricted regimens, blood samples were taken at about eight-week intervals for calcium, phosphorus and magnesium determinations. A total of 99 samples were collected during the four-year period. The analyses were within the normal range and failed to show anything that could be traced to variations in the calcium-phosphorus ratio of the diets.

An average of all of the samples with averages of the first three and last three samples taken for each of the horses are presented in table IV. The calcium content remained at about the same level in all of the samples throughout the experimental period. There was a reduction in the phosphorus as the animals advanced in age, which is physiologic, but it was more marked in some animals than in others.

Bone Analyses

When the marked bone changes were found to exist, chemical analyses were made to determine if normal mineralization existed.

In table II the data from these analyses are presented for horse 1 and filly 4. These data show the total ash, calcium and phosphorus as well as the calcium carbonate, tricalcium phosphate ratio to be normal. Despite this finding, the bones of horse 1 were so light compared with those of filly 4 that further investigations were made. This reduction in weight was due to failure of normal mineralization of the Haversian systems (fig. 2) and reduced thickness of the compact bone of the shaft in the long bones (fig. 1). Even more significant was the marked cancellation of the entire length of the compact bone, and this was equally well marked in the short bones, such as the phalanges, and the membranous bones, such as the patella.

Figure 1 shows a cross section of the cannon bones and a sagittal section of the distal end of the femurs of these two horses. The bone changes so marked in horse 1 were not again demonstrated in any of the remaining animals in the experiment. This animal's mate, filly 2, kept in the same corral on the same rations, had very slight alterations in the quality of the bone. On the other hand, joint lesions were common to all of the animals.

We are, therefore, not in a position to attempt a satisfactory explanation of the development of this advanced bone alteration in horse 1.

On account of variations in age of these horses and size of their bones it was necessary to ascertain their water displacement in order to obtain an accurate idea of the variations in density. These va-

TABLE IV—Calcium, Phosphorus and Magnesium Analyses of Blood of Experimental* and Normal† Horses

Horse Number	Blood Calcium in Mg.%			Blood Phosphorus in Mg.%			Blood Magnesium in Mg.%		
	Average of All Calcium Determinations	Average of First Three Samples	Average of Last Three Samples	Average of All Phosphorus Determinations	Average of First Three Samples	Average of Last Three Samples	Average of All Magnesium Determinations	Average of First Three Samples	Average of Last Three Samples
Horse 1	12.18	12.02	11.65	5.39	6.65	3.62	2.97	3.01	2.85
Filly 2	12.00	11.92	11.57	5.76	6.76	5.62	3.38	3.14	4.12
Horse 3	12.82	12.51	13.35	4.64	5.83	2.53	2.45	2.15	1.95
Filly 4	12.39	12.11	12.36	4.93	5.50	4.01	2.45	2.42	2.77
Horse 5	12.10	12.21	12.49	5.48	6.03	5.32	2.93	3.10	2.96
Horse 6	12.73	12.66	12.65	5.30	6.17	5.22	2.83	3.15	—
Filly 7	13.23	13.36	13.75	4.09	5.69	3.85	2.55	2.57	2.78
Horse 8	12.83	12.65	13.28	4.42	5.74	3.68	2.26	2.78	2.18
Filly 10	12.68	12.33	12.44	4.56	5.45	4.76	2.23	2.69	1.69
Horse 11	13.25	13.12	14.53	4.63	5.43	4.40	2.36	2.07	2.52
Normal	12.85	12.63	13.37	4.78	5.12	4.10	2.95	2.96	2.87

*Total 99 samples.
†Total 83 samples, from horses of comparable ages in the farm stud.

tions were great between horse 1 and all others examined. The bones were painted with liquid paraffin to prevent the entrance of water so that this could be demonstrated. These data are presented in table III.

In rationing these animals, it was desired to supply all the feed they would clean up, which varied considerably from time to time. The straw supplied to the first series of horses was not weighed. The rations given to these horses were not chemically analyzed because the bone and joint alterations had not been anticipated. Vitamin A was the real factor under study. Barley straw grown on or in the vicinity of the University Farm is low in calcium, varying from .02 to .08 per cent. The concentrates given were weighed and recorded each day.

In table I for the first series of horses while straw was being fed the Ca-P ratios have been figured on an average intake of 10 lb. of straw and 13 lb. of concentrates daily. The straw was estimated from other analyses to contain .05 per cent calcium and .01 per cent phosphorus. The concentrates fed to these animals were estimated to contain .11 per cent calcium and .70 per cent phosphorus.

Concentrates were fed alone to the second series at the average rate of 17 to 19 lb. daily and the latter figure used in calculating the ratio. Diets 3, 4 and 5 were analyzed for calcium and phosphorus. Thus, the widest calcium-phosphorus ratio diets fed to horse 1 and filly 2 in the first series and horses 5 and 6 in the second series varied more in daily intake of phosphorus than in calcium, the actual average daily intake being about 8.6 Gm. of calcium and 41.6 Gm. of phosphorus in the first two animals as compared with 8.6 Gm. of calcium and 61.1 Gm. of phosphorus for the second two animals. The use of the calcium-phosphorus ratio is only an approximation at best. Availability is involved, particularly in the light of recent work tending to show the low absorbable phytic acid phosphorus, which constitutes a considerable part of the phosphorus in seeds, including cereal grains.

Discussion

The interpretation of the data obtained from these eleven experimental animals presents difficulties.

All of the animals except horse 9, which died from encephalomyelitis 58 days after going on the experiment, developed symptoms of vitamin A deficiency and this deficiency was allowed to progress until it was the primary cause of their death. All of the animals except horse 9 showed characteristic joint lesions. Horse 6, group C was killed *in extremis* after being on experiment 286 days without night blindness having been definitely demonstrated. The testing for this symptom was interfered with because of severe lameness. This animal was given carotene treatment in the feed from January 17 to March 6, 1939.

A total of 375 cc. of cocoanut oil to which had been added 14 mg. of carotene per cubic centimeter was given over the period. Some improvement in the lameness was noticed for a time. This was temporary, however, and it soon became difficult for the animal to rise even with help and it was destroyed on March 7. The 250 blue units of vitamin A per gram of liver tissue found *post mortem* could be accounted for from the carotene treatment. This animal had grown rapidly and had marked joint lesions.

This case casts some doubt as to the etiologic relationship of vitamin A deficiency to the joint lesions in the animals because of the failure of the carotene treatment over the last 48 days of life to arrest the condition. A somewhat similar situation occurred in horse 1, but this case was too far advanced before the cod liver oil treatment was started.

The possibility of the rations being deficient in some other known or unknown dietary essentials was recognized. In order to eliminate vitamin C deficiency, horses 7, 8 and 11 were given 400 mg. of ascorbic acid daily from October 13, 1939, to the date of death, covering periods of 169, 186 and 240 days, respectively. To ascertain if more B complex than that in the rations was needed, horses 7 and 11 were given, in addition to the ascorbic acid, 4 oz. daily of brewers' yeast during the last 71 and 179 days, respectively, of their lives. These additions to the diet made little change in the animals and had no effect on the terminal result and lesions found.

Because vitamin A lesions are so definitely manifested in epithelial tissues, the attributing of joint lesions in tissues of mesoblastic origin to this cause must be done with discretion. Curative treatments in these cases would have been inconclusive because of the chronic nature of joint lesions and the lack of definite indices to recognize their incidence during life. The lack of joint lesions in the foals of fillies 4 and 7 can not be explained.

Two of the animals were carried on rations having a calcium-phosphorus ratio of 1 to 5, and two others with this ratio at 1 to 7. Only one of the animals, horse 1, showed marked skeletal alterations. Filly 2, three months younger, on the same ration at the same time except that straw was not fed during the last 102 days, and in the same corral had no comparable bone alterations. The bones of horses 5 and 6 receiving the same calcium-content ration with the wider ratio were comparable with those of filly 2. Thus it would appear that marked individual alteration occurs in this species and that a 1:7 ratio is not a serious factor in preventing normal calcium-phosphorus utilization and skeletal development. The rather wide ratio may or may not have been responsible for the individual exception. Horse 1 had ample sunlight and sun-bleached straw in its ration, which, we feel, could eliminate a vitamin D deficiency. In other respects its environment was identical with the remaining animals. There was a reduction in blood calcium in this animal, but the average of the last three samples was higher than the same samples of filly 2. The blood phosphorus became markedly reduced during the experiment, but still was not so low as that of horse 3, and about equal to that of horses 7 and 8.

The difficult breathing in several of these animals was identical and became so marked that in the weakened condition of the animals it was the principal immediate cause of death. It is interesting in the light of Mitchell's hypothesis attributing the cause of roaring to vitamin A deficiency.

The experimental animals were under such conditions in small corrals and having joint complications that exercise to demonstrate hemiplegia was not obtainable. The marked dyspnea was due to a typical paraplegia. Only horse 11 was observed to make a whistling respiratory sound in a few respirations on the day of his death. The data provide good evidence that vitamin A deficiency in horses in a considerable percentage of cases affects the movements of the larynx and paraplegia is a frequent terminal symptom.

The symptoms of atypical stringhalt and shivering manifested by filly 10 were not sufficiently clear cut for us to discuss them further. The prolonged and excessive

flexion of the hind legs may have been caused by the pain from the joint involvement and the apparent evidence of shivering may have been the result of extreme weakness. The latter gradually developed in all of the cases.

The reproductive processes in this species as in the others studied continue to function until the deficiency is far advanced, but gestation is affected. Filly 4 became pregnant and died in foaling. Filly 7 gave birth to one abnormal foal at term and died at the end of the second gestation period. These animals were bred by horses 3 and 8, respectively. Horse 3 impregnated filly 4 about 30 days before he demonstrated night blindness and she became night blind after gestation had progressed about six months. Horse 8 impregnated filly 7 the first time shortly after they were started on experiment and both were normal. Foaling occurred about 3½ months before she was night blind. The foal was unable to nurse and its liver was negative for vitamin A. This dam was again impregnated by horse 8 on May 10, 1939, 17 days after she foaled. Horse 8 was night blind first on January 16, 1939, and although he received minimum levels of cod liver oil for a time, he was night blind when this fertile mating occurred. The mare became night blind August 8, 1939, three months after impregnation. She was given treatment with minimum requirements of cod liver oil or carotene, was again night blind November 17, 1939, and died March 30, 1940, with the foal fully developed *in utero*. She had thus carried the foal for a period of eight months after night blindness first developed and during the last 117 days of life was continuously night blind.

Sexual libido of horses 8 and 11 was manifested until they were far advanced in the deficiency. On postmortem examination the testicles were found to be soft and flabby. Sections of the testicles of horse 8 showed marked shrinking of the seminiferous tubules and great increase of interstitial cells which produce the male sex hormone. Fillies 2 and 10 did not become pregnant even though seen to breed repeatedly to horses 1 and 9, respectively. Filly 2 also was bred by horse 3.

The eye symptoms in these cases were characteristic as in the other species studied so far as the night blindness was concerned. Typical xerophthalmia as sometimes seen in cattle did not develop, but there was keratinization of the cornea manifested by irregular opaque streaking which appeared to be an infiltration within the layers, rather than the surface being affected. In the most advanced cases the eyes were partially closed and the infiltration extended, making the entire cornea cloudy so that examination of the deeper structures of the eye with the ophthalmoscope was difficult and the animal manifested a dull, listless appearance.

One eye from each of three of the experimental animals—filly 2 and horses 3 and 6—with a normal eye from a control animal were sent to Dr. Katherine Tansley, University College, London, for study of the retinal changes. On account of war conditions this study has not been completed.

None of these cases developed any evidence of permanent blindness from pinching off of the optic nerves in the optic canals as has been commonly found in young cattle. This may have been due to their age when deficiency was manifested, but no naturally occurring cases of this condition in horses have been reported.

The growth curves of these horses (fig. 5) show considerable variation. This was in part due to vitamin A therapy. The curves of filly 4 and horses 5 and 11 show that a fatal termination can occur without great weight loss.

Conclusions

The data on these eleven horses indicate that the progressive condition leading to death from vitamin A deficiency shows a quite characteristic syndrome in this species. Night blindness, lacrymation, keratinization of the cornea, respiratory symptoms, reproductive difficulties, capricious appetite, progressive weakness and death occur quite regularly.

Accompanying these symptoms in all

cases there developed joint involvement with lameness and characteristic rarefying lesions in the joint cartilages.

Positive evidence that the joint condition was due primarily to the vitamin A deficiency was not obtained, but the evidence pointed to the possibility of this being the case.

Varying the calcium-phosphorus ratio from 2 to 1 to 1 to 7 in the diets did not seem to play an important rôle in the nutrition of the animals. The advanced bone alterations in horse 1, if due to this cause, were evidence of marked individual variation.

Rations commonly fed to horses where yellow corn is not available are liable to be deficient in vitamin A.

Swine Erysipelas, The Agglutination Test for Its Diagnosis, and a Report on a Study of Arthritis in Swine*

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SINCE OUR FIRST report in 1932 on the agglutination test for swine erysipelas, continued studies have been made on this problem. Improvements in the preparation of the antigen and the application and interpretation of the test have been given particular study. Since the antigen, as now prepared, differs considerably from that originally reported, it would seem desirable to give the technic of the preparation of this product.

A smooth colony culture of *Erysipelothrix rhusiopathiae* known to produce satisfactory antigen[1] is maintained in either beef extract or beef-infusion broth, pH 7.4, and subcultured once a week. Two-liter (2.1-qt.) flasks, containing 1-liter (1.05-qt.) amounts of beef broth adjusted to a pH of 7.4, are inoculated with 0.5-cc. amounts of a 24-hour growth of the smooth *E. rhusiopathiae* in broth of the same type suitable for antigen production. Before seeding, the flasks of media should be warmed in the incubator for 24 hours.

The inoculated flasks are incubated at 37.5° C. for 24 hours. Usually eight flasks are seeded for each lot of antigen. Ten cc. of the 40 per cent solution of formaldehyde is then introduced into each flask and returned to the incubator for an additional 24 hours. The organisms are then harvested by means of supercentrifugation with a Sharples centrifuge. The organisms are collected from the centrifuge by using a spatula or a small brush and as small an amount of the collecting fluid as is necessary to recover all of the organisms.

The collecting fluid consists of physiologic saline solution to which has been added sufficient formalin to constitute a 1 per cent solution. The concentrated antigen is then agitated and stored in the dark at room temperature for two weeks with occasional agitation during this period. This allows maximum lysis of the cells to take place before final adjustment of the density is made. After two weeks of storage the density of the antigen is adjusted to correspond to 50 X No. 1 of the McFarland scale.

Necessary dilution is made with formolized saline solution (collecting fluid). The antigen is allowed to stand several days until the organisms have settled sufficiently so that 20 per cent of clear, supernatant liquid can be pipetted off, discarded and replaced with an equal amount of sterile C. P.

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[1]Schoening, H. W., Creech, G. T., and Grey, C. G.: A laboratory tube test and a whole blood rapid agglutination test for the diagnosis of swine erysipelas. No. Amer. Vet., xiii (Dec. 1932), pp. 19-25.

glycerin. Finally, 2 per cent of a saturated aqueous solution of crystal violet is added. The antigen is then complete and ready for use.

TECHNIC OF THE TEST

Either whole blood or serum can be used. The reaction can best be seen when a white porcelain plate is used. Wire loops of 3/16 of an inch in diameter are made at the end of convenient lengths of nichrome wire, gauge No. 24, which have been calculated to hold about .02-cc. amounts. Antigen is measured by means of a standard medicine dropper which should deliver a drop of .05 cc. when held in a vertical position.

In making the test a drop of antigen is placed on the plate and a full drop of blood or serum, as the case may be, is collected in the loop and mixed with the drop of antigen on the plate in the usual manner of conducting any plate test. It is important that the drop of blood or serum be a full one; in other words, it should be the maximum amount of blood or serum that can be caught on the loop.

The plate is then rotated and reading made within a two-minute period. In positive cases a marked clumping of the bacteria occurs usually within less than a minute, and in most cases within two minutes. We feel that the test should not be considered positive unless a definite clumping occurs within the two-minute period. If clumping does not occur within this period, reaction is considered negative. As a control, one drop of the antigen, smeared to the same size as the test proper, should be carried out from time to time, and the reactions observed in the test should be compared to the antigen alone.

Known positive and negative serum also should be tested from time to time to check the specificity of the antigen. The antigen has been found to be quite stable and a large number of tests conducted have indicated it to be specific.

In the diagnostic use of the test, it is quite important to place proper interpretation on the results of the test and to recognize its limitations. It is believed that a definitely positive reaction to the test in swine indicates either infection with or exposure to the specific organism. It is possible that positive reactions may be obtained in animals which show no clinical evidence of the disease. The reactions in these animals may be explained on the basis that swine-erysipelas organisms may be harbored in the body of the animal, such as the tonsils or intestinal tract, without producing any visible lesions or symptoms of the disease.

It is also possible that there may be obscure joint lesions in an animal, the result of erysipelas infection. Since erysipelas manifests itself very frequently by invasion of the joints, many animals that react may have obscure joint lesions which can be detected only by a very thorough postmortem examination of all of the joints. Other animals that react may simply be harboring the organisms as before mentioned in the tonsils or possibly the intestinal tract. It is for this reason that caution should be exercised in interpreting the positive results to the test.

Generally speaking, the test is considered more applicable to a herd diagnosis rather than to the diagnosis of the disease in individual animals. Where erysipelas is suspected in a herd, a number of animals should be tested and diagnosis not made until a representative serologic picture of the herd can be obtained. It has been our experience that animals showing joint lesions visible on clinical examination react strongly.

It also has been noted that animals treated with swine-erysipelas antiserum give a strong reaction, even as early as 24 hours following treatment, and this reaction may persist for some weeks. The test, therefore, has little value in animals which have recently received specific antiserum. Animals in the acute stage of the disease may give a positive reaction, but this is quite variable. The fact also should be kept in mind that the chronic form of swine erysipelas may be in a herd which may be affected with some other acute disease, so that while a positive reaction to swine erysipelas might be obtained, it would not necessarily indicate that some

other infection is not also present in the herd. This fact always should be kept in mind.

It has been found that normal horse and cow serums may agglutinate the antigen. This is not considered to be of any importance, since it is thought to be nonspecific, unless the serum reacts in very high dilutions.

ARTHRITIS

The organisms of swine erysipelas seem to have a marked predilection for the joints. This disease in the acute form is septicemic, the organisms are found in the blood, and very early, even in this stage of the disease, joint involvement may take place. Arthritis may be seen as the result of an acute attack of swine erysipelas or as a result of a low-grade infection in which the animal may not be noticeably sick at any time, but may appear later with enlarged joints.

The relationship between the low-grade type of infection and acute outbreaks of the disease is not known, but it is probable that under certain circumstances an acute outbreak of the disease may suddenly appear on a farm which had had only a low-grade type of infection present.[2] Upon incising and exposing the involved surfaces of arthritic joints caused by swine erysipelas, a rather typical picture is presented, depending upon the length of time that the disease has been present.

In the early stages there may be only a slight inflammation of the synovial membranes. With the persistence of the infection, granulation tissue forms and connective tissue proliferation gives rise to the formation of so-called synovial fringes, either attached to the synovial membranes or freely suspended in the joint fluid. As a result of the chronic inflammation, the capsular ligaments become greatly thickened. The articular surfaces may show erosions. Osteitis and periosteitis may be observed at times.

A study of arthritis in swine in specimens submitted from a number of the meat-inspection centers was undertaken by the federal bureau of animal industry in 1939. While it was definitely demonstrated by Ward[3] in 1922 that many cases of arthritis in swine are due to erysipelas infection, it was thought advisable to obtain additional information on this subject since it would give some idea as to the distribution of the disease in various parts of the country. Specimens of arthritis in swine were, therefore, obtained from a number of meat-inspection centers in various parts of the country along with information as to the origin of the hogs from which the specimens were obtained. The collection of specimens was begun in February 1932. The following report concerns the specimens collected from that time to date.

A total of 472 joints were received and examined bacteriologically. The swine-erysipelas organism, *E. rhusiopathiae*, was recovered from 357 of these, a percentage of 75.6. The swine from which positive results were obtained had their origin in 91 counties in the following 14 states: Iowa, Nebraska, Minnesota, South Dakota, Texas, Oklahoma, Arkansas, Tennessee, Mississippi, Alabama, Florida, Virginia, Maryland and Pennsylvania. While 75 per cent of the specimens examined yielded the erysipelas organism, it is quite probable that this percentage would have been higher had it been practical to make extensive cultural tests of all the affected joints.

SUMMARY

A description is given of the preparation of the antigen for the plate agglutination test for swine erysipelas. An interpretation of the test and its limitations are discussed.

Of 472 arthritic joints from swine which were examined bacteriologically, 357, or 75.6 per cent, yielded *Erysipelothrix rhusiopathiae*. These specimens originated in 91 counties in 14 states.

[2]Schoening, H. W., Peterman, J. E., and Grey, C. G.: A study of an outbreak of acute swine erysipelas in one herd. J.A.V.M.A., xcvi (June 1940), pp. 714-718.

[3]Ward, A. R.: The etiology of polyarthritis in swine. J.A.V.M.A., lxi (1922), p. 155.

The Relative Efficiencies of Disinfectants in Killing Erysipelothrix Rhusiopathiae*

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THE IMPORTANCE of *Erysipelothrix rhusiopathiae* to veterinarians is emphasized by the occurrence of swine-erysipelas infection in practically every section of the United States and the recent recognition of this organism as a pathogen of turkeys, ducks, quail and lambs as well as of swine and man. Practicing veterinarians frequently are asked to suggest methods of eradicating the infection from premises following an epizoötic. They also should know which disinfectant provides the greatest margin of safety when used on the hands following exposure to this organism.

Although the official phenol coefficients of most disinfectants are well known; and most disinfectants now carry a statement of the phenol coefficient upon the label, the phenol coefficient can not represent the value of a disinfectant against all types of organisms and under all conditions. This is due to the fact that the official phenol coefficient is a comparison of the dilution of the disinfectant, with the dilution of phenol, which is required to kill *Eberthella typhosa* in broth at 20° C. (68° F.) when ten parts of the dilution of the disinfectant are mixed with one part of the culture.

Obviously, such data may yield little information as to the comparative values of disinfectants against organisms unrelated to *E. typhosa* and in the presence of organic matter such as feces.

The authors are of the opinion that the basis for the selection of the product best suited to accomplish disinfection under the conditions it will be used, and against a given organism can only be the result of comparative tests with that organism under conditions closely parallel to those of actual application. Such data can be obtained solely by means of large numbers of tests, and the gaps in the information available will be filled in but slowly. As the results of such tests are published, the practitioner of veterinary medicine must add considerable specific information to his professional knowledge. The selection of the disinfectant best suited to destroy a given type of infection under specific conditions requires no less professional knowledge and is no less important than the selection of a therapeutic agent.

REVIEW OF THE LITERATURE

Although *E. rhusiopathiae* was observed by Loeffler in 1882 and by Thuillier in 1883, the literature contains but little critical data which can provide bases for the selection of the disinfectants best suited for its destruction. When the problem is further complicated by the practical necessity of the disinfection being accomplished in the presence of small amounts of manure, such as escape removal in the ordinary cleaning of barns and pens, the information is even more meager.

Petri is quoted[1] as saying the following disinfectants are effective against *E. rhusiopathiae*: chlorate of lime 1 per cent, hot lye 1 per cent, soda 5 per cent, iron sulfate 3 per cent, copper sulfate .25 per cent, bichloride of mercury .10 per cent, carbolic acid, creolin and lysol.

Nederveen[1] found that antiformin destroyed the organism in 15 minutes but did not dissolve it in ten days, while Blum and Herrenheiser are quoted[1] as saying, "Salvarsan does not kill them either *in vivo* or *in vitro*."

Van Es and McGrath[2] state that the virulence of pure cultures disappeared only after 26 days in strong brine while the organisms in hams treated with salt and saltpeter retained their virulence for at least 30 days and those in the pickling fluid were still virulent after 170 days. They also claim destruction of the organism by four hours of exposure to formaldehyde vapor

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[1]Hutyra, Marek and Manninger: Special Pathology and Therapeutics of the Diseases of Domestic Animals (4th Eng. ed., Baillière, Tyndall and Cox, London, 1938), I, p. 78.

[2]Van Es, L., and McGrath, C. B.: Swine erysipelas. Univ. of Nebraska Agr. Exp. Sta. Res. Bul. 84 (1936), pp. 6-7.

and by 15-minute exposures to solutions of phenol and saponified cresols, while 1 per cent hypochlorate of lime, 0.1 per cent mercuric chloride, .25 per cent copper sulfate, and 1 per cent hot lye solutions were also effective in varying lengths of time.

A 0.1 per cent concentration of the bile salt, sodium glycocholate, *in vitro* was found to be bactericidal and a similar concentration of sodium taurocholate rendered cultivation difficult, but .0001 per cent of the two salts in the broth medium enhanced growth of the organism.[3]

TECHNIC

Three virulent cultures of *E. rhusiopathiae* were used.* These were found to be practically identical in resistance. The technic for testing disinfectants described by the Food and Drug Administration[4] was followed with only such modifications as were necessary for tests with *E. rhusiopathiae*. In one series of the trials a sterile suspension of pig manure was added to provide additional organic matter and thus more nearly to parallel conditions of actual disinfection.

The cultures to be mixed with the disinfectant for testing were grown in F.D.A. broth[4] which was autoclaved and cooled just previous to inoculation. This expelled sufficient of the dissolved oxygen to allow good growth of the organism, and in all trials in which the data are included, growth in subculture followed the inoculation of one-millionth of a loop. Dilutions of one ten-millionth of a loop usually failed to initiate growth. With the F.D.A. technic this would indicate that each loop subcultured after exposure to the disinfectant contained the survivors of between 100,000 and 1,000,000 viable inocula. Subcultures were made into a semisolid medium consisting of Difco peptone 10 Gm., beef extract 5 Gm., salt 5 Gm., agar 1.5 Gm., dextrose 3.5 Gm., sufficient brom-thymol-blue to tint, and 1,000 cc. of distilled water. This was alkalinized to give a final pH of 7.3. The strains of *E. rhusiopathiae* grew well in this medium and produced sufficient acid to give a readily recognizable change in the color of the brom-thymol-blue.

As in the F.D.A. method, one part of culture was mixed with 10 parts of disinfectant and held in a water bath at 20° C. (68° F.). Subcultures were taken at the end of 5, 10 and 30 minutes. These were incubated at 37° C. (98.6° F.) at least seven days before being discarded.

The suspension of pig feces, of which 1 cc. was added to 10 cc. of the disinfectant in those tests where additional organic matter was supplied, was made by adding an equal volume of water to fresh, well-formed pig feces. This was autoclaved 30 minutes, strained through sugar sacking to remove large particles, bottled, autoclaved and stored in the ice box until used. The final reaction was pH 6.74; upon standing 48 hours approximately 30 per cent of its volume was semisolid material which had settled out. One hundred cc. of this mixture weighed 102.14 Gm.; when evaporated to dryness, this yielded 7.53 Gm. of dry residue, and after incineration 2.7 Gm. of ash remained. This material was well mixed before being added to the disinfectant. It is assumed that the addition of 10 per cent of this suspension is equivalent to the addition of 5 per cent of fresh pig feces.

EXPERIMENTAL DATA

Phenol in broth, with an exposure of ten minutes, killed *E. rhusiopathiae* in a dilution of 1:90, which is approximately the same dilution of phenol as is known to kill *E. typhosa* when the F.D.A. method is used.[4] The presence of the suspension of pig manure had but little effect upon the germicidal efficiency of phenol solutions. When applied in the recommended concentrations of 2 to 5 per cent, phenol is a reliable disinfectant against this organism. However, the cost of such concentrations of phenol, together with its other undesirable characteristics, such as toxicity to animals in the concentrations which are germicidal, and its strong odor which may prohibit its use near milk or eggs, make it one of the less satisfactory disinfectants against this organism. Its germicidal powers are known to be adversely affected by cooling and by dilution, which further detracts from its reliability under field conditions.

Liquor cresolis compositus killed *E. rhusiopathiae* in dilutions of 1:666 to 1:1,200 in broth and in dilutions of 1:250 to 1:300 when pig manure was present. As compared with phenol, it is obvious that the presence of particulate organic matter greatly decreased the germicidal powers of liquor cresolis compositus. Like phenol it

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*The authors are indebted to Dr. A. S. Rosenwald of the Oregon State College for two of the cultures used.

[3]Kolda, J.: The influence of bile on the swine erysipelas bacillus. Rev. Gén. Méd., xxxiii (1924), pp. 293-312; quoted from Exp. Sta. Rec., lii (1925), pp. 283-284.

[4]Ruehle, G. L. A., and Brewer, C. M.: United States Food and Drug Administration methods of testing antiseptics and disinfectants. U. S. Dept. Agr. Circ. 198 (1931).

is adversely affected by cooling and by dilution and has an odor which is absorbed readily by food products.

Lysol, which has a phenol coefficient of five against *E. typhosa*, has practically the same germicidal efficiency under the conditions of these tests as does liquor cresolis compositus, to which it is related.

Aseptogen killed in dilutions of from 1:666 to 1:1,666 in the absence of added organic matter and in dilutions of 1:166 to 1:250 in the presence of the pig manure.

Formalin, which is a 40 per cent solution of formaldehyde gas, was found to be very inactive. This confirms work previously reported[5] in which it has been found that at 20° C., in dilutions greater than 1:14 and 1:15, it did not kill *E. typhosa* in ten minutes. In the presence of the pig manure, a 1:20 dilution was required to kill *E. rhusiopathiae* in ten minutes and a 1:25 dilution in 30 minutes. A 1:100 dilution failed to kill in the absence of added organic matter and greater concentrations were not tried. These data are sharply in contrast with the usual opinion of formaldehyde as a disinfectant, although they do not differ significantly from the data previously submitted by a number of bacteriologists who have made critical tests and who have differentiated between bacteriostasis and bactericidal action. Scott[6] found that 1:200 formaldehyde, which is equivalent to 1:80 formalin, killed all aerobic bacteria, both spore-forming and non-spore-forming, in six to twelve hours and the sporulating anaerobes in from two to four days. The greater time of exposure in these trials probably accounts for the efficiency of less concentrated solutions.

The germicidal efficiencies of formalin solutions are influenced by moderate changes in the temperature more than are the efficiencies of other disinfectants in common use. It has been shown[5] that against *E. typhosa*, in the absence of added organic matter, reducing the temperature from 20° C. to 2° C. increased the concentration of formalin necessary to kill from 1:15 to 1:7.4, while at 40° C. a dilution of 1:130 killed. In the presence of added organic matter, which in this case was a suspension of human feces, the effective dilutions at 2° C., 20° C. and 40° C. were 1:6.7, 1:14 and 1:92.5, respectively.

The much higher germicidal efficiency reported for formalin by numerous workers, whose data provide the bases for the recommendation of this product as a germicide, may have been based upon the absence of visible growth after the usual period of incubation of the subcultures. Thus, if the subcultures had been read only after one day, it would have appeared that 2 per cent formalin killed in five minutes and that 1 per cent or less killed in 10 or 30 minutes. If the subcultures had been read only after two days, it would have appeared that 3 per cent killed in five and in 10 minutes; while 2 per cent killed in 30 minutes. Incubation of the subcultures for 7 days revealed that 6 per cent was not sufficient to kill in five or 10 minutes and 4 per cent was required to kill with an exposure of 30 minutes (table I).

From these data, it is obvious that formalin can not be considered an effective disinfectant against *E. rhusiopathiae*.

Lye* killed in dilutions of from 1:500 to 1:1,200 in the absence of added organic matter and in dilutions of 1:333 to 1:500 in the presence of 5 per cent pig manure. The high-test household lye used contained approximately 98 per cent sodium hydroxide, which previously was reported to be germicidally active against *Brucella abortus*[7] and *E. typhosa*[5] in the presence of organic matter. The germicidal efficiency of sodium hydroxide solutions has been shown to be but little affected by moderate changes of temperature. The data indicate household lye, which can be pur-

[5] McCulloch, E. C., and Costigan, Stella: A comparison of the efficiency of phenol, liquor cresolis, formaldehyde, sodium hypochlorite and sodium hydroxide against *Eberthella typhi* at various temperatures. J. Inf. Dis., lix (1936), pp. 281-284.

[6] Scott, J. P.: The action of phenol and formol on aerobic and anaerobic organisms. J. Inf. Dis., xliii (1928), pp. 90-92.

*Lewis lye, not less than 97.5 per cent sodium hydroxide, furnished by the Pennsylvania Salt Manufacturing Company, was used in these trials.

[7] McCulloch, E. C.: The germicidal efficiency of sodium hydroxide. J. Bact., xxv (1933), pp. 469-493.

TABLE I—Results with Formalin

Concentration of Formalin	Subcultures Read After	Time of Exposure to Formalin								
		5 Min.			10 Min.			30 Min.		
		1	2	7	1	2	7	1	2	7 Days
6%		—	—	+	—	—	+	—	—	—
5%		—	—	+	—	—	+	—	—	—
4%		—	—	+	—	—	+	—	—	—
3%		—	—	+	—	—	+	—	—	+
2%		—	+	+	—	+	+	—	—	+
1%		+	+	+	—	+	+	—	+	+

— = Absence of growth in subcultures.
+ = Growth in subcultures.

chased for 10 cents a can, to be the disinfectant of choice against *E. rhusiopathiae*. It is odorless and the dilution of 1:150, which now is recommended, is not toxic to animals when consumed in moderate amounts, nor is it markedly caustic to the skin.

Drene, said to be triethanolammoniumlauryl sulfate, previously was found[8, 9] to be markedly effective against mastitis streptococci. In the absence of added organic matter, drene killed *E. rhusiopathiae* in dilutions of 1:500 to 1:666; in the presence of 5 per cent pig manure, it killed in dilutions of 1:166 to 1:200. While this product now is being marketed as a shampoo, its low toxicity to skin and high germicidal efficiency against certain pathogens indicate that it, and products of related chemical composition, may have value in the disinfection of the hands after exposure to *E. rhusiopathiae*. The possibility is suggested that some product of like nature be supplied to the veterinary profession through the usual commercial channels. Other work[10] indicates, however, that triethanolammoniumlaryl sulfate possesses little germicidal activity against Gram-negative organisms, such as *E. coli*, *E. typhosa* and *Br. abortus*.

[8]McCulloch, E. C.: Soaps as disinfectants. Proc. Washington State Public Health Assn., v (1939), pp. 39-45.

[9]McCulloch, E. C.: The efficiency of soaps and other disinfectants in destroying mastitis streptococci. Amer. J. Vet. Res., i (Oct. 1940), pp. 18-22.

[10]Cowles, P. B.: A modified lactose broth for use in the presumptive test. J. Amer. Water Works Assn., xxx (1938), pp. 979-980.

Soap likewise was found to exert germicidal action against the swine-erysipelas organism, killing in dilutions of from 1:100 to 1:110 in the presence of the 5 per cent pig manure. The prophylactic value of thoroughly washing the hands with soap and warm water after having been in contact with this organism should be emphasized. The soap used in these tests was ivory soap in bars, but additional experiments indicate that other brands of household soaps possess approximately the same germicidal efficiency.

Lugol's solution (5 Gm. of iodine and 10 Gm. of potassium iodide in 100 cc. of water) killed in dilutions of 1:1,250 and 1:1,666 in the absence of added organic matter and in dilutions of 1:166 and 1:200 in the presence of 5 per cent pig manure. The apparent anomaly will be noted in figure 2 that in the presence of the pig feces, subcultures taken from the 1:200 dilution of Lugol's solution at the end of five and ten minutes failed to initiate growth, while

TABLE II—Survival of E. Rhusiopathiae in Salt Solutions

Concentration (Per Cent)	Exposure in Days			
	88	94	122	171
30	Alive	Dead	Dead	Dead
24	Alive	Dead	Dead	Dead
18	Alive	Dead	Dead	Dead
12	Alive	Dead	Dead	Dead
6	Alive	Dead	Dead	Dead
3	Alive	Alive	Variable	Variable

subcultures taken at the end of 30 minutes sometimes were viable. This phenomenon was observed in three trials. A possible explanation is that during an exposure of five or ten minutes to this concentration

HIGHEST DILUTION KILLING ERYSIPELOTHRIX RHUSIOPATHIAE IN THE ABSENCE OF ADDITIONAL ORGANIC MATTER AT 20°C. (68°F).

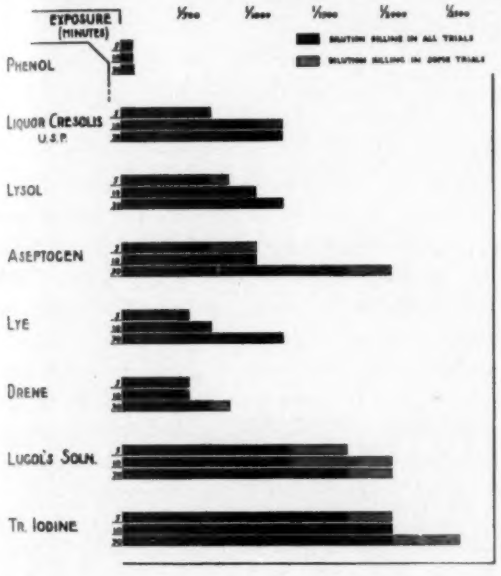

Fig. 1.

of Lugol's solution, sufficient iodine became loosely attached to the bacterial cells to prevent multiplication when they were transferred to the subculture medium. During the longer period in contact with the suspension of pig feces and Lugol's solution, the pig feces may have combined with the iodine in the Lugol's solution to form less toxic compounds and some of the iodine loosely attached to the bacterial cells may have undergone similar changes. Tincture of iodine killed in dilutions of 1:1,666 to 1:2,000 in the absence of additional organic matter and in a dilution of 1:250 when 5 per cent pig manure was present.

Chlorine gas can be used as a disinfectant only where elaborate and expensive equipment is available. The hypochlorites are most widely used as farm disinfectants. The chlorine solutions used in these tests were made by dissolving a commercial hypochlorite powder in water. The product used contained 50 per cent "available chlorine" and was of comparatively low alkalinity. The resulting solutions were mixtures of sodium and calcium hypochlorites. In the absence of added organic matter, from 80 to 120 parts of "available chlorine" per million killed. This would correspond to dilutions of 1:12,500 to 1:8,000 of chlorine or dilutions of 1:6,250 to 1:4,000 of the com-

HIGHEST DILUTION KILLING ERYSIPELOTHRIX RHUSIOPATHIAE IN THE PRESENCE OF 5% PIG FECES AT 20°C. (68°F).

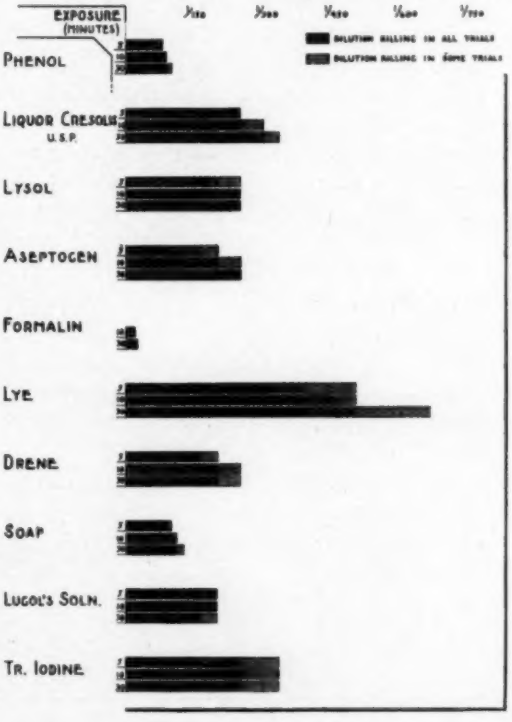

Fig. 2.

mercial hypochlorite powder. In the presence of the suspension of pig feces, from 80 to 90 parts of "available chlorine" per million killed. This would correspond to dilutions of from 1:12,500 to 1:11,000 of the chlorine. Here again an apparent anomaly occurs. Chlorine solutions are known to be affected adversely by the presence of even small amounts of organic matter. However, chlorine solutions become much more actively germicidal as the de-

TABLE III—The Highest Dilutions of Disinfectants Killing E. Rhusiopathiae in broth at 20° C. (68° F.)

	Highest Dilutions Lethal			Comparative Efficiencies
	5 Min.	10 Min.	30 Min.	
Phenol	1:90	1:90	1:100	1.0
Drene	1:500	1:500	1:666	5.5
Lye	1:500	1:666	1:1,200	7.4
Liquor cresolis	1:666	1:1,000	1:1,200	11.1
Lysol	1:666	1:1,000	1:1,200	11.1
Aseptogen	1:666	1:1,000	1:1,666	11.1
Lugol's solution	1:1,250	1:1,250	1:1,666	13.9
Tr. iodine	1:1,666	1:2,000	1:2,500	22.2
Chlorine	1:8,000	1:10,000	1:12,500	111.0

gree of alkalinity is reduced.[11] In order to obtain a reasonable degree of stability, all hypochlorites must be prepared with a rather high degree of alkalinity. It is possible that when the solution of the commercial hypochlorite was mixed only with the broth and culture, a slight degree of alkalinity remained. In the presence of the more highly buffered suspension of pig feces, which was slightly acid in reaction, it is probable that the final mixture was approximately neutral in reaction. The technical difficulties in determining the hydrogen-ion concentration of hypochlorite solutions made it impractical to demonstrate this experimentally.

Alcohol exhibited some ability to kill *E. rhusiopathiae* but provided only a scant margin of safety. With an exposure of five minutes, in the presence of 5 per cent pig manure, 60 per cent ethyl alcohol killed in all trials; 50 per cent sometimes killed and sometimes failed; 40 per cent was not effective. With an exposure of ten minutes, 60 per cent was the most dilute which killed in all trials, although both 50 per cent and 40 per cent alcohol sometimes killed. When allowed to act for 30 minutes, 50 per cent alcohol consistently killed, 40 per cent killed sometimes and 30 per cent had no apparent effect upon the organisms. Since an exposure of even five minutes to the alcohol solution would practically never be used in disinfecting skin, alcohol does not provide a reasonable margin of safety when applied to the skin after exposure to this organism.

[11] Charlton, D. B., and Levine, M.: Some observations on the germicidal efficiency of chloramine-T and calcium hypochlorite. J. Bact., xxx (1935), pp. 163-171.

TABLE IV—The Highest Dilutions of Disinfectants Killing E. Rhusiopathiae in the Presence of 5 Per Cent Pig Feces at 20° C. (68° F.)

	Highest Dilutions Lethal			Comparative Efficiencies
	5 Min.	10 Min.	30 Min.	
Hydrogen peroxide 3 per cent solution	Failed	Failed	1:5	
Alcohol	60%	60%	50%	
Formalin		1:20	1:25	0.2
Phenol	1:80	1:90	1:90	1.0
Soap	1:100	1:100	1:110	1.1
Lugol's solution	1:200	1:200	1:166	2.2
Drene	1:166	1:200	1:166	2.2
Aseptogen	1:166	1:200	1:200	2.2
Tr. iodine	1:250	1:250	1:250	2.8
Lysol	1:200	1:250	1:250	2.8
Liquor cresolis	1:250	1:250	1:300	2.8
Lye	1:333	1:450	1:500	5.0
Chlorine	1:11,000	1:12,500	1:14,000	139.0

Hydrogen peroxide solution, as offered to the medical profession, usually consists of a 3 per cent aqueous solution of H_2O_2. In the presence of the 5 per cent suspension of pig manure the full-strength 3 per cent, as used undiluted, failed to kill in five of six trials. With an exposure of ten minutes, the 3 per cent solution killed. Increasing the time of exposure to 30 minutes resulted in killing in all trials with 0.6 per cent solution of hydrogen peroxide, obtained by mixing one part of the commercial solution with four parts of water. A 0.3 per cent solution of hydrogen peroxide gave irregular results. It is apparent that washing the skin for one or two minutes with the commercial solutions of hydrogen peroxide can not be relied upon to kill *E. rhusiopathiae*.

Salt solutions (table II) were tested for their effect upon *E. rhusiopathiae* because the survival of this organism in brines and in salted meats may have an important bearing upon its dissemination. The temperature of the refrigerator in which the cultures were held in the brines varied from —1.8°C. (30°F.) to 4.4° C. (40° F.).

These data indicate that, at low temperature, *E. rhusiopathiae* is able to survive in concentrated brines for approximately three months. It is interesting to note that in 1892, Freytag[12] was quoted as having written, "The bacillus of Rouget (French swine plague) survives salt solutions for two months."

SUMMARY

Erysipelothrix rhusiopathiae is readily destroyed by household lye (sodium hydroxide) and the hypochlorites. Where it can be used, household lye is the most practical disinfectant. Phenol, liquor cresolis and related products, Lugol's solution, tincture of iodine, triethanolammoniumlauryl sulfate and household soaps also are moderately effective. *E. rhusiopathiae* is resistant to formaldehyde, hydrogen peroxide, alcohol and salt brines.

[12]de Freytag, C. J.: The action of common salt on bacteria. Arch. f. Hyg., xi, p. 60; quoted from Bacteriological World and Modern Medicine, i (1891), p. 92.

Correction (on "The Relation of Brucellosis to Periodic Ophthalmia in Equidae," by T. C. Jones): In the October 1940 issue of the American Journal of Veterinary Research (volume I, number 1), page 54, column 2, paragraph 3, in the sentence, "It will be noted in table I, that agglutinations occurred in very few of the sera in dilutions that are high enough to be of positive diagnostic significance (1:800 or higher)," the figure 1:800 should read 1:80.

Laboratory Tests of the Potency of Antirabic Vaccines*

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THE STUDY of virus diseases is still in that early stage where there are few fixed principles of general applicability and where a reasonable understanding of a disease requires in consequence the accumulation of many empirical facts. Usually these facts can be established only through experiments based on many animals. Great progress in the study of a virus disease, therefore, ordinarily follows the discovery of a suitable test animal. Webster's recent work on rabies in mice is important because it has called to attention the possibilities that lie in the exploitation of this animal as a test object.

It is unnecessary to point out to the present audience the desirability of increased scientific knowledge of rabies and the urgent need of that better control which may reasonably be expected to result from it. We all know that success in controlling this disease depends upon the extent to which it is limited in dogs. There are two ways of effecting this limitation. The first and essential way is through an adequate policing which will restrict the spread of infection by muzzling and by limiting the range of licensed dogs and by the thorough-going destruction of all strays. The second way is through a widespread vaccination which will raise the general level of resistance to infection of the canine population. This discussion is devoted to aspects of the latter approach.

When one considers the problems of canine vaccination, two types of questions arise. One has to do with the potencies of the vaccines available for use, that is (a) with the development of methods for demonstrating and then for assaying vaccine potency and (b) with attempts to produce better vaccines. The second question is concerned with establishing a vaccination procedure that, with the available vaccines, will convey an acceptable immunity to vaccinated dogs. A final and satisfactory answer to this second question obviously depends upon a sufficient knowledge of vaccine potency. For this reason, we have concentrated attention upon the first question, the evaluation of vaccine potency.

POTENCY TEST SELECTED

As one phase of his study Webster examined the ability of various commercial vaccines to protect mice. He found that though a few of these products would protect against many lethal doses of virus, no immunizing power whatsoever could be shown for many of them. Proceeding from this observation we undertook a thorough investigation of the immunizing capacity of all batches of vaccine made by one manufacturer. Our first task was the selection of a potency test simple and cheap enough for the routine and large-scale application we had in mind. Webster's work set the general outlines of such a test but for our purposes it was essential to define certain conditions more closely and it was important that the time consumed by the test, as well as its cost, be kept at a minimum. Before deciding upon a uniform test whose conditions would be followed in all future research work, we studied the influence of a number of factors, therefore, such as age of mouse, number of and interval between vaccinations, interval between first vaccination and challenge-virus injection, etc. These preliminary studies convinced us that it is not hard to prepare killed vaccines that will protect most mice against many lethal doses of intracerebral virus; they also led us to adopt as the basis for our future work a test[1] carried out under the following conditions:

Duration of test: Twenty-six days.

Mouse: Our experiments have been con-

*Presented before the first general session of the 77th annual meeting of the AVMA, Washington, D. C., August 26-30, 1940.

†Lederle Laboratories, Inc.

[1]Wyckoff, R. W. G., and Beck, C. E.: J. Immunol., xxxix (1940), p. 17.

TABLE I—A Comparison of Degree of Immunity Conferred on 3- and 6-Week-Old Mice

Vaccine No.	Vaccinations	Survivals 14 Days After Virus Injection									
		Virus Dose (mld.) (10 Days After 1st Vaccination)					Virus Dose (mld.) (14 Days After 1st Vaccination)				
		1	10	100	1,000	10,000	1	10	100	1,000	10,000
		3-Week-Old Mice									
76V262A	4 daily	5/5	5/5	3/5	3/5	0/5	5/5	5/5	4/4	2/4	2/5
76V262A	6 daily	4/4	4/4	3/3	2/4	2P/4	4/4	4/4	3/4	2/4	2/5
76V263A	4 daily	5/5	4/4	4/5	4P/5	5P/5	5/5	5/5	4/5	1/5	4/5
76V263A	6 daily	4/4	4/4	1/4	1/4	1/4	4/4	4/4	3/4	3/4	4/5
		6-Week-Old Mice									
76V262A	4 daily	5/5	5/5	4/5	4/5	1/5	5/5	5/5	4/5	4/5	3/5
76V262A	6 daily	5/5	5/5	5/5	5/5	3/5	5/5	5/5	4/5	2/5	3/5
76V263A	4 daily	4/4	5/5	5/5	3/5	3/5	5/5	5/5	5/5	3/5	3/5
76V263A	6 daily	5/5	5/5	4/5	4/5	2P/5	5/5	5/5	5/5	4/5	4/5

Note: The two vaccines listed in this table were chloroform-killed, for canine use.

In this and the following tables the number before the dash is the number of survivors, the number after the dash the number of vaccinated mice receiving virus. Thus, 2/4 means that two out of the four mice receiving virus survived. As mentioned in the text, some vaccinated mice became paralyzed after virus injection without succumbing to disease. Such mice have been marked P in the tables.

ducted principally with the "caracul" strain of mice bred by the Carworth Farms, but we have done enough to show that other strains of "Swiss" mice are equally satisfactory. We routinely use mice 3 weeks old at the beginning of the test.

Vaccine dose: A volume of 0.6 cc. of a 1:9 dilution of vaccine. Dilution is made just before use, the diluent being saline.

Number of vaccinations: Five at daily intervals.

Mode of vaccination: Intraperitoneal.

Date of challenge-virus injection: Twelve days after the first vaccination.

Route of virus injection: Intracerebral.

Virus inoculum: For routine testing usually 100 and 1,000 lethal doses (mld). Volume of inoculum, .03 cc. Source of virus: infected rabbit brain.

Number of vaccinated mice on each virus dilution: Usually five.

Date of reading test: Two weeks after virus injection.

Unless otherwise stated, the results to be reported later in this paper have been obtained with this test. I do not wish to lay any claim to especial value upon the precise conditions we have chosen—equally significant results could be obtained from other variants of the mouse test—but I do wish to emphasize the importance of choosing some precisely defined test and of following it implicitly. Unless this is done it is impossible to get results of real significance.

The kind of results observed with this test when mice are vaccinated with reasonably good commercial canine vaccines are shown in tables I and II. These happen to have been collected during the preliminary investigation of the effect on the resulting immunity of increasing the number of vaccinations. They are typical of a large mass of similar data.

Barring Worthless Vaccines from Sale Would Help to Restore Confidence in Rabies Vaccination

Having settled upon a routine test, we applied it to many lots of freshly made human and veterinary vaccines of one manufacturer designated L. Results of one group of such tests typical of the others are listed in table III. It will be noted that while most lots produce a well-defined immunity, there is a certain percentage which is devoid of immunizing power. We are convinced that the first practical step

TABLE II—A Comparison of Immunity Developed 10 and 14 Days After First Vaccination

Vaccine No.	Vaccinations	Survivals 14 Days After Virus Injection									
		Virus Dose (mld.) (10 Days After 1st Vaccination)					Virus Dose (mld.) (14 Days After 1st Vaccination)				
		1	10	100	1,000	10,000	1	10	100	1,000	10,000
76V262A	3 daily	4/4	4/5	4/5	0/5	2P/5	5/5	3/5	0/5	0/5	1/5
76V262A	4 daily	5/5	5/5	3/5	4/5	5P/5	4/4	5/5	5/5	4/4	4/4
76V262A	6 daily	5/5	5/5	4/4	3/5	5/5	5/5	5/5	4/4	5/5	5/5
76V263A	3 daily	5/5	3/5	2/5	0/5	0/5	5/5	4/4	3/5	0/5	0/5
76V263A	4 daily	5/5	3/5	3/5	2/5	5P/5	5/5	4/4	5/5	5/5	4/5
76V263A	6 daily	5/5	5/5	5/5	5/5	5/5	5/5	5/5	——	4/5	5/5

Note: The two vaccines listed in this table were chloroform-killed, for canine use. The mice used in this test were 3 weeks old at the time of first vaccination.

to be taken in improving the quality of rabies vaccines is routine testing of all vaccine lots and enforced rejection of all weak and worthless batches; and we are convinced that the elimination from sale of demonstrably worthless vaccines would do more than anything else to restore confidence in the value of canine rabies vaccination. The testing itself is not expensive and the fact that such "flat" batches do not represent more than about 15 per cent of the total production means that it would be feasible to restrict the sale of vaccines to those of proved and tested potency without unduly raising their cost.

Assuredly Potent Vaccine Not Available

After we had obtained in this way an idea of the quality of these vaccines, we tested some vaccines of other manufacturers. Typical results are assembled in table IV. Some of these vaccines, such as D2 and E2, are among the best that we have ever examined; many of the others have had no demonstrable potency. It was not a part of our purpose to do so, and we have not tested enough other commercial vaccines to know what proportion of the output is weak, but I want to point out that D and E, manufacturers of excellent

TABLE III—A Comparison of the Immunizing Capacity of Different Lots of Fresh Vaccine of Maker L

Vaccine	Type of Vaccine	Survivors 14 Days After Virus Injection Virus Dose (mld.)	
		100	1,000
I	Human (phenol-killed)	5/5	5/5
II		1/5	0/5
III		3/5	0/5
IV		1/5	1/5
V		2/2	4/5
VI		4/5	3/5
VII		5/5	1/3
1	Veterinary (phenol-killed)	4/5	3/5
2		0/5	0/5
3		2/2	3/3
4		3/5	2/5
5		5/5	2/5
6	Veterinary (Chloroform-killed)	5/5	5/5
7		4/4	3/4

TABLE IV—Immunizing Power of Several Manufacturers' Vaccines (as Challenged with the L Strain of Fixed Virus)

MAKER	LOT NO.	SURVIVORS FROM VIRUS DOSE OF		
		1 MLD	10 MLD	100 MLD
A	1	0/5	0/5	0/5
	2	1/5	1/5	0/5
B	1	1/5	0/5	0/5
	2	2/5	0/5	0/5
	3	——	3/4	3/5
	4	——	3/5	2/4
C	1	——	0/5	0/4
	2	1/5	0/4	0/4
D	1	——	5/5	3/5
	2	——	4/5	4/5
	3	2/5	0/5	0/5
E	1	——	1/5	0/5
	2	5/5	5/5	5/5
F	1	——	4/4	2/5
	2	1/5	0/5	0/5

products, are selling at the same time the impotent vaccines D3 and E1. In other words, as far as we can determine no manufacturer is now offering an assuredly potent vaccine; each would enhance the quality and reliability of his vaccines through routine testing and the elimination of worthless batches.

QUANTITATIVE STANDARDS OF IMMUNIZING WORTH ARE DIFFICULT TO ESTABLISH

This first step—eliminating impotent vaccines—would be relatively simple to accomplish. The next step—setting up definite quantitative standards of immunizing worth—is much more complex. We have carried out experiments designed to supply some of the information upon which such standards must be based, but our data are still incomplete. All commercial vaccines are presumably made from "fixed" rabies virus—virus which has been cultivated for many years by serial passage through the brains of laboratory animals, usually rabbits. It is commonly assumed that fixed virus is modified by its prolonged laboratory cultivation with the result that it has lost affinity for the peripheral nerves and has thus lost more or less its capacity to produce a fatal rabies in animals except when injected intracerebrally or in massive doses. There can be no real doubt of this difference between fixed and "street" virus and, since there are fixed viruses having different origins and histories, we must admit the likelihood that these fixed viruses, too, differ from one another. There thus arises the possibility that vaccines made with different viruses, though alike in their capacities to protect animals from the viruses from which they were prepared, may vary widely in the protection they offer against other strains of rabies virus. This possibility must be thoroughly explored before satisfactory standards of vaccine potency can be set up. To provide data bearing on this point we have studied the degree of immunity developed by vaccination when that immunity is challenged by injection of the virus from which the vaccine was made (homologous virus) and when other strains of rabies

TABLE V—Potencies of an L Vaccine When Challenged by Heterologous Strains of Virus

VACCINE	VIRUS	SURVIVORS FROM VIRUS DOSE OF			
		1 MLD	10 MLD	100 MLD	1,000 MLD
76V263	L	5/5	5/5	4/5	2/5
	NIH	5/5	5/5	2/5	3/5
(5 vac)	CSA	4/5	0/5	0/5	——
	JF	3/4	0/5	——	——
76V263	L	——	5/5	5/5	5/5
	NIH	5/5	5/5	5/5	——
(10 vac)	CSA	5/5	3/5	4/5	——
	JF	5/5	4/4	——	——

TABLE VI—Potencies of Several Good Vaccines When Challenged by Heterologous Strains of Virus

MANUFACTURER	VIRUS	SURVIVORS FROM VIRUS DOSE OF			
		1 MLD	10 MLD	100 MLD	1,000 MLD
L	L	5/5	4/5	4/5	2/5
	NIH	5/5	3/3	3/5	——
	CSA	5/5	3/5	1/5	0/4
	JF	3/5	4/5	——	——
E	L	5/5	5/5	5/5	3/4
	NIH	5/5	4/5	3/4	——
	CSA	5/5	4/4	5/5	3/5
	JF	5/5	5/5	——	——
D	L	——	5/5	3/5	5/5
	NIH	5/5	5/5	3/5	2/5
	CSA	5/5	0/5	0/5	——
	JF	5/5	3/5	2/5	——

Note: Ten daily vaccinations.

virus were injected instead (heterologous viruses).

Vaccines by manufacturer L were used in the first experiments of this sort. The challenge viruses employed in this and all the experiments to be described later were the following: 1) The L strain of fixed virus, from which the vaccine was made; this virus, an offshot of the original Pasteur strain, was imported from Paris by the New York City board of health and obtained from the board by the manufacturer many years ago. 2) The National Institute of Health's fixed virus* (NIH), which also is a descendant of the Pasteur strain but which has had a different history during the many years it has been under cultivation in this country. 3) A "semi-fixed" strain (CSA) originating in a street virus isolated fairly recently and carried since then through many generations in the brains of mice. 4) A street virus (JF) isolated a few months ago from a rabid dog and passed through a restricted number of generations in mice. In their properties there are minor, but apparently real, differences between the two fixed strains; the differences between these fixed strains and the other two less adapted viruses are clear-cut. Our early experiments indicated that L vaccines capable of giving a good immunity against the L virus from which they were made would also provide a well-defined protection against NIH fixed virus. The protection against CSA virus was definitely poorer while that against the JF street virus, though genuine, was too small to permit quantitative comparisons between vaccines. In the hope of raising this protection against street virus we tried the effect of making ten instead of the five daily vaccinations called for in our test. As the data of table V indicate, this increase in amount of administered vaccine does improve materially the immunity to street and street-like virus.

In our next experiments several commercial vaccines were similarly tested against these four viruses. With the exception of certain products of one manufacturer, vaccines which showed up badly against L virus in the tests already described were no more effective against the other viruses. Three good commercial vaccines are compared in table VI. There seems little to choose among them. All protect well against the fixed strains and, with the exception of vaccine D in relation to virus CSA (which should be confirmed by further study before too much meaning is attached to it), they yield a clearly defined immunity to street-like virus.

These results have direct bearing upon the question of absolute standards of vac-

*We are indebted to Karl Habel of the National Institute of Health for samples of this and of the CSA virus described.

cine potency. The real criterion of potency, of course, is and always must be the ability to protect against street virus. But there are serious objections to the routine use of street virus for such testing. It is less infectious and less regularly infectious for mice than adapted virus and it has a prolonged and irregular incubation period that adds to the difficulties of quantitative work with it. The danger of human infection is great and this makes it hard to work with routinely. Furthermore, we are not yet sure that all street viruses are exactly alike and we must always remember that as ordinarily carried in the laboratory a freshly isolated strain soon loses many of its street-like characteristics.

Ability to Protect Against Fixed Virus Identifies Potency of Rabies Vaccine

We believe that all the evidence thus far obtained indicates that, except for the purposes of research, the potency of rabies vaccines can be adequately gauged from their ability to protect against fixed virus. If this is accepted, it raises the question of what strain of fixed virus is to be taken as standard. As far as we know, there is no indication that one strain is preferable in this respect to another. Until such evidence is forthcoming we believe it best, as it is easiest, to express the potency of a rabies vaccine in terms of its ability to protect againt the (homologous) virus used in making it. We do not know what minimal standards of vaccine potency in terms of homologous virus protection should be set; scientifically sound ones can be arrived at only after extensive vaccination tests on dogs in which carefully tested vaccines are used. In the meantime, however, our experience indicates that it is unnecessary to sell or use vaccines so weak that when tested according to the conditions outlined above they can not protect vaccinated mice against at least 100 mld of homologous virus.

Improving Potency Hinges on Knowledge of Keeping Qualities

We now come to the third major question with respect to rabies vaccines, namely, what can be done to improve their potency once worthless vaccines are eliminated and acceptable standards are set for vaccine potency? The procedure is clear. First, it is imperative to acquire knowledge of the keeping qualities of vaccines. This can be done with the help of the mouse test, with vaccines of adequate initial potency. We are conducting such experiments with one set of vaccines; in the course of time we will know how long they retain their potency in the ice box, at room temperature and at summer heat.

Problems in Manufacture

Questions as to the best way to manufacture killed rabies vaccines are complex and will take a long time to answer. One of the first answers needed is whether or not one strain of fixed virus gives a more

TABLE VII—Potencies of Experimental Vaccines When Challenged by Various Strains of Virus

Virus for		1 mld	10 mld	100 mld	1,000 mld
Vaccine	Challenge				
L	L	5/5	5/5	5/5	3/5
	NIH	5/5	4/5	4/4	—
	CSA	5/5	5/5	1/5	1/5
	JF	4/5	0/5	—	—
NIH	L	2/4	3/5	2/5	1/5
	NIH	3/5	2/4	1/4	—
	CSA	4/4	1/3	0/4	—
CSA	L	5/5	5/5	2/4	2/4
	NIH	3/4	5/5	5/5	—
	CSA	5/5	5/5	3/5	3/5
	JF	4/5	2/5	—	—

potent vaccine than another. We have carried out experiments that throw some light upon this point. Following a uniform procedure several experimental phenolated vaccines have been made with both the L and the NIH fixed viruses and with the CSA adapted street virus. Mice vaccinated with these vaccines have been tested for immunity to each of these viruses and to our JF street virus. A few of the results are shown in table VII. The CSA and L vaccines are better than the NIH vaccine, but the difference is quantitative rather than qualitative and not outside the normal variations found between different batches of vaccine made with the same virus.

Sometimes one hears it claimed that one kind of rabies vaccine is superior to another because it is made with an "antigenically superior" strain of virus, one inherently capable of producing a more potent vaccine. We have not experimented with enough strains to disprove such a statement, but it must be said that all the evidence we have thus far assembled points to the fact that the details of the manufacturing process are far more important for the production of a potent vaccine than is the choice of a special strain of fixed virus. It should be borne in mind, however, that different strains probably vary sufficiently in such important details as their inactivation rates so that the optimum conditions for preparing a vaccine with one strain are not necessarily identical with those required for another strain. We are investigating for one fixed virus the effects on potency of variations in manufacturing procedures. We hope that these studies will help to establish more clearly the details of preparation which determine whether a batch is "good" or "flat."

GENERAL CONSIDERATIONS

There are various other problems of interest to the users and makers of rabies vaccines that are now amenable to scientific study. Thus it is important to know what relation there is between the virus content of the tissue from which vaccine is made and the potency and stability of the resultant vaccine. It is also important to study, particularly with reference to vaccines for human use, the properties of purified and concentrated vaccines and to determine whether they offer any real advantages over the present cruder vaccines. These and similar questions are intimately related to the fundamental one of what is the nature of the immunizing principle in killed rabies vaccines. It is highly important to both the manufacture of vaccines and to the development of the best mode of vaccination to know whether this active material is a noninfectious residuum of the living virus or the total virus modified and killed by the inactivating agent. At present arguments can be adduced in favor of either hypothesis. At this point I wish merely to say that with the mouse test new experiments can be brought to bear on this question. It is only a matter of a short time before we will be in position to discuss this question with greater profit.

SUMMARY

I hope that what I have said has convinced you that an active program of research on rabies will greatly enlarge our knowledge of the vaccines to be used in its prevention and control. Summarizing again the practical aspects of our results to date we can say:

1) It is possible to define a mouse test of vaccine potency that is simple and inexpensive enough for routine and large-scale use.

2) With such a test it is feasible to control the potency of all lots of manufactured vaccine.

3) Good commercial vaccines convey a powerful and readily demonstrable immunity to mice, but a large amount of vaccine now on the market is too weak to give a substantial immunity to mice.

4) There is no scientific or economic reason for the continued sale and use of such impotent vaccines. We believe that the general adoption of a mouse test to assure that all marketed vaccines have a demonstrable potency is the most important step that can be taken to improve the quality of rabies vaccines and to raise confidence in this important means of bringing a dangerous disease under better control.

5) For the production of a potent vaccine

Congenital Defect of the Interventricular Septum, Aortic Regurgitation and Probable Heart Block in a Dog*

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CONGENITAL abnormalities of the heart have been noted in domestic animals, such as transposition of the large arterial trunks, stenosis of the pulmonary orifice, defects of the auricular and interventricular septa, right aortic arch, pericardial defects and valvular anomalies. However, relatively few cases have been recorded in dogs. Aellig,[1] Lallengen[2] and Olafson[3] have reported a total of 25 congenital cardiac abnormalities in these animals of which four were isolated interventricular septal defects. The dog of Hare and Orf[4] had a patent ductus arteriosus in addition to the septal defect.

The dog which is the subject of this report was an 18-kg. (40-lb.) brown female Setter 6 to 7 years of age, healthy and sound in appearance. The right carotid artery had been cannulated for experimental purposes under light ether anesthesia. Several hours were permitted for the effects of the anesthesia to wear off. Pulse and respiration rate were 36 and 52 per minute, respectively. Rectal temperature was 38.5° C. Palpation revealed a typical water-hammer pulse. The results of various chemical tests were as follows: arterial blood CO_2 27.8 and O_2 9.7 volumes per cent; venous blood CO_2 30.8, O_2 6.13 and O_2 capacity 11.2 volumes per cent; serum proteins 5.6 Gm. per cent, and serum Cl 700 mg. per cent; red blood cells 3.18 millions; hemoglobin (Sahli) 60 per cent; blood volume, cells 24, and plasma 76 per cent. The arterial blood pressure was measured by means of a Tycos anaeroid gauge with a T tube connected on one side to the cannulated carotid artery and to a citrate pressure bottle on the other. It was found to be 210/50. A blood-pressure tracing was then taken by means of the hypodermic manometer of Hamilton[5] and the pressure was found to be 265/25 (fig. 1). Immediate autopsy was performed.

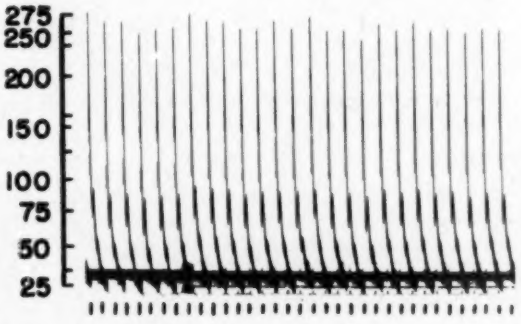

Fig. 1. Blood-pressure record with Hamilton manometer. Femoral artery. No anesthetic. Abscissa, time in seconds; ordinate, blood pressure, calibrated in millimeters of mercury. (Taken by Miss E. Lindner, Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.)

POSTMORTEM EXAMINATION

Aside from the findings in the heart, the pathologic diagnosis was chronic passive

*From the departments of pathology and gastro-intestinal research of the Michael Reese Hospital; this study was aided by a grant from the A. B. Kuppenheimer fund.

[1]Aellig, A.: Foramen interventriculare persistens cordis in domesticated animals. Schweiz. Arch. f. Tierh., lxxii (1930), p. 509; lxxiii (1931), p. 10.

[2]Lallengen, K.: Hjärtmissbildningar hos djuven. Skand. Vet.-Tids., xxiv (1934), p. 493.

[3]Olafson, P.: Congenital cardiac anomalies in animals. J. Tech. Meth., xix (1939), p. 129.

[4]Hare, T., and Orf, A. B.: Patent ductus arteriosus with patent interventricular foramen of a dog's heart. J. Path. & Bact., xxxiv (1931), p. 799.

[5]Hamilton, W. J., Brewer, G., and Bratman, I.: Pressure pulse contours in the intact animal. I. Analytical description of a new high-frequency hypodermic manometer with illustrative curves of simultaneous arterial and intracardiac pressures. Amer. J. Physiol., cvii (1934), p. 427.

(Continued from preceding page)

a well-understood and carefully controlled manufacturing process is the important factor, rather than the use of a special strain of fixed virus.

hyperemia of the liver and spleen, acute hepatitis and chronic pyelonephritis with contraction of the kidneys.

Heart (figs. 2 and 3).—The heart was enlarged and weighed 175 Gm. (6 oz.). The apex was formed by the left ventricle. From the base two arteries emerged, one anterior and to the left, and the other posterior and to the right. The mutual relationships of the various heart chambers were normal.

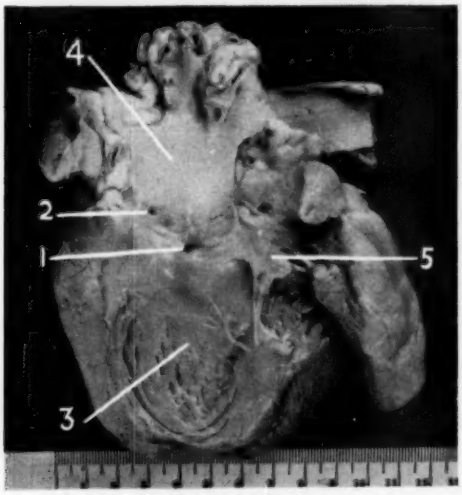

Fig. 2. Left ventricular view of the heart. (1) Defect in the pars membranacea, (2) right coronary ostium, (3) muscular interventricular septum, (4) aorta, (5) mitral valve.

The right auricle was normal in size. The superior and inferior vena cava and coronary sinus entered this chamber normally. The limbus was well formed and the foramen ovale was closed. The orifice and leaflets of the tricuspid valve and the corresponding chordae tendineae and papillary muscles were normally formed and showed no remarkable changes.

The chamber of the right ventricle was larger than normal and its wall measured 2 mm. in thickness. The pulmonary artery emanated from this chamber. The orifice and cusps of its valve were normally formed and presented no pathologic change. The two pulmonary arteries were given off normally. The ductus arteriosus was closed. The topography of the muscle bundles of the right ventricle was normal, but they were flattened as were the columnae carneae.

The left auricle was normal in size. It received the four pulmonary veins normally. The septum primum was well formed. The mitral orifice was normal in size. The mitral valve leaflets were normally formed, but their edges and corresponding cordae were somewhat thickened.

The left ventricular chamber was distinctly enlarged and its wall measured 10 mm. at the base and 4 mm. at the apex. The papillary muscles were flattened. The muscular interventricular septum was normally formed; the pars membranacea, however, presented an elliptical defect the larger horizontal diameter of which measured 7 mm. The pars membranacea elsewhere was irregularly thickened throughout with a nodular, grayish yellow, plaque-like formation on its inferior border. The center of the defect lay beneath the commissure between the right and posterior aortic cusps. The adjacent portions of the cusps were pulled downward by the thickened edges of the defect. The aortic ostium was dilated. The aortic cusps were well formed but slightly thickened at their edges. At the base of the aorta above the right sinus of Valsalva, an elevated, grayish yellow plaque 4 mm. in diameter was seen. The coronary ostia and arterial distribution presented no changes.

Fig. 3. View of the base of the aorta. Note the small defect in the pars membranacea (arrow) and the thickened and retracted aortic valve cusps.

Histologic Study of the Bundle of His (figs. 4, 5, 6).—The muscle fibers of the proximal part of the bundle of His stain irregularly. Some show a clear-staining, homogeneous, markedly eosinophilic cytoplasm with pyknotic nuclei. Others show the more usual granular pole-staining cytoplasm with normal nuclei. As it courses through the pars membranacea beneath the defect in the interventricular septum, the bundle is almost completely replaced by acellular fibrous and fat tissue. The fibers that still remain show degenerative changes. Similar degenerative and fibrotic changes are noted in the main branches of the bundle. A large artery which courses through the upper part of the muscular interventricular septum is almost completely occluded by a markedly thickened intima. Smaller arteries are similarly thickened. There is a focal increase in connective tissue through the musculature of the septum, with degenerative changes less marked than in the bundle of His.

Cardiac Diagnosis:

1) Patency in the interventricular septum in the region of the pars membranacea.

2) Fibrosis in the region of the pars membranacea with retraction of the bases of the posterior and right anterior aortic leaflets, and resultant aortic insufficiency.

3) Arteriosclerosis with narrowing of the arteries of the muscular interventricular septum.

4) Degenerative changes and fibrosis of the bundle of His and its branches.

5) Hypertrophy and dilation of the left and right ventricles, predominantly the left one.

DISCUSSION

Clinically the points of interest in this dog were the evidence of aortic regurgitation, the slow pulse, the high systolic blood pressure and the anemia.

The clinical evidence of aortic regurgitation was well explained pathologically by the unusual findings of a retraction of the bases of the posterior and right anterior aortic cusps. The constant flow of blood through the septal defect evidently produced a thickening (fibrosis) of the mar-

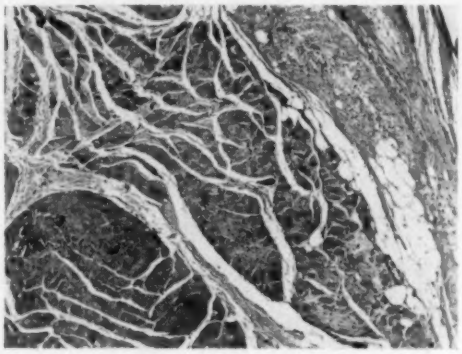

Fig. 4. Proximal portion of the bundle of His. The muscle fibers stain irregularly. The cytoplasm of some stains deeply eosinophilic, and the nuclei are pyknotic. In the lower portion there is some replacement by fibrous tissue. (Hematoxylin-eosin, x 44.)

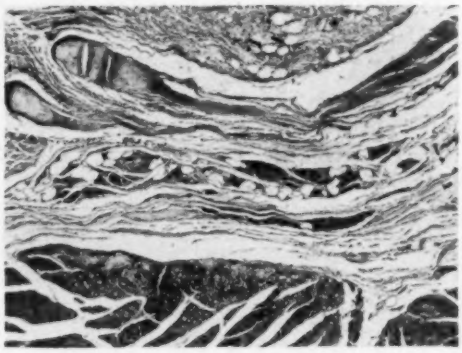

Fig. 5. Bundle of His in the region of the defect of the pars membranacea. The bundle is almost completely replaced by fibrous tissue. The muscle fibers still present show degenerative changes. (Hematoxylin-eosin, x 44.)

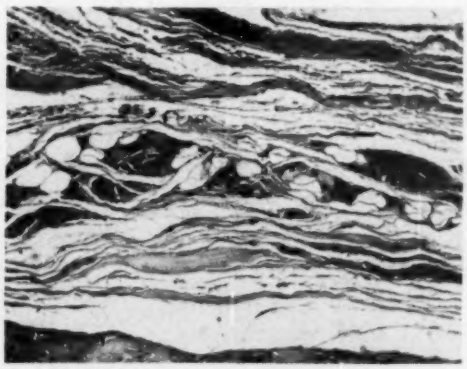

Fig. 6. High power of figure 5. (Hematoxylin-eosin, x 88.)

gins with the consequent retraction of the adjacent bases of the aortic cusps. This may be due to the fact that the pars membranacea is relatively small in the dog, the aorta practically taking its origin from the muscular interventricular septum. The aortic cusps could thus easily be retracted by a fibrotic process in the small pars membranacea. One would not expect such a complication of a septal defect in the human heart because of the relatively larger pars membranacea.

A possible explanation of the slow pulse lay in the degenerative and fibrotic changes in the bundle of His; we were dealing most likely with a complete heart block, although unfortunately no electrocardiographic study was made to confirm this. Due to the constant increase in fibrous tissue at the base of the pars membranacea, the changes in the bundle occurred. This was probably aided by the ischemia produced by the arteriosclerosis of the septal arteries.

We do not know whether the hypertension could be ascribed to the pyelonephritis. Although there were marked changes in the vascular tree in the region of the pyelonephritic scars, the remainder of the kidneys showed minimal vascular changes. If it is the production of an ischemic kidney which accounts for the hypertension at times found in pyelonephritis, histologic evidence was insufficient to warrant that ischemia had been produced.

The rather high degree of anemia present in this animal which appeared outwardly healthy and lively may perhaps be due to the pyelonephritis and to the hepatitis.

The explanation of the genesis of the defect in the interventricular septum is dependent upon the interpretation of the method of formation of the pars membranacea in the mammalian heart. The various theories have been reviewed elsewhere (Lev and Saphir[6]). Such a defect is most probably due to a lack of fusion of the endocardial cushions with the bulbar swellings and the muscular interventricular septum. Which of these elements is directly responsible for this lack of fusion is conjectural. The rarity of reports of isolated interventricular septal defects in dogs may be ascribed to the small size of the pars membranacea in these animals.

[6]Lev, M., and Saphir, O.: Congenital aneurysm at the membranous septum. Arch. Path., xxv (1938), p. 819.

A Comparison of Flotation Solutions in the Detection of Parasite Ova in Feces*

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IN 1878, Grassi and Parona[1] pointed out that hookworm infection could be diagnosed by the finding of the ova of the worms in the feces. They used the simple smear method for examination, which was the only one used for many years. Later many investigators, in their search for better methods in the detection of worm eggs in feces, mainly in connection with hookworm infection in man, devised many variations in technic and materials. Some of the methods were slight variations of the simple smear; others were elaborate methods requiring considerable apparatus and material.

One of the first modifications of the simple smear method was the sedimentation method. This consisted in mixing water with the fecal material, allowing the mixture to stand for a short time and then examining for ova by spreading the sediment on slides. By this method large amounts of fecal material could be used because the heavy material could be decanted, leaving just a small amount of sediment with the ova. A variation in the sedimentation method was the use of a centrifuge to concentrate the material more rapidly and, therefore, lessen the time in examination.

Hall[2] wrote that "undoubtedly the feature of fecal examination which has been most intriguing and enticing to investigators is that of flotation." In this method a solution is used with a specific gravity higher than that of the ova to concentrate them on the surface of the fluid. The ova are then removed with the surface film to a microslide either indirectly with a wire loop, or directly by placing a microslide on top of the container, with the solution touching the slide. Later the centrifuge was used to concentrate the ova on the surface more rapidly and again lessen the time in examination. The ova were removed from the surface film as in the indirect flotation, but later workers, in a method known as the direct centrifugal flotation method, placed a cover slip directly on the top of the tube so that the ova would adhere to the cover slip.

MATERIALS AND METHODS

In discussing fecal flotation, Hall[2] wrote, "With a well-stocked chemistry laboratory or the household pantry available there appears to be almost endless possibilities in the number of substances which may be tested in work along this line." In reviewing the literature, the writer has found this to be true, for many different solutions have been used by many workers, each praising the efficiency of his own solution and method. The object, therefore, of this test was to compare some of the solutions recommended and to find a solution that has a specific gravity sufficiently high to concentrate the greatest number of parasite ova on the surface of the fluid, without causing their rupture. The five solutions used were sodium nitrate, zinc sulfate, magnesium sulfate, sodium chloride and cane sugar.

Saturated sodium nitrate solution was prepared by adding 850 Gm. (1 lb., 12 oz.) of the salt (sodium nitrate C.P., cryst.) to one liter (1.05 qt.) of distilled water at room temperature. This was shaken vigorously, and left standing for 24 hours to allow the salt to dissolve. Twenty-five to 50 Gm. of salt was then added so that there always was some undissolved salt in the bottom of the container. Repeated tests were made with a hydrometer to assure accuracy of the specific gravity, which, as reported by Kalantarjan,[3] is 1.400.

The zinc sulfate solution contained 1,000 Gm.

* From the Department of Veterinary Parasitology, College of Veterinary Medicine, The Ohio State University.

[1] Bibliography of hookworm disease. Pub. No. 11, International Health Board, Rockefeller Foundation, New York, p. xiii.

[2] Hall, M. C.: Developments in veterinary parasitology during the past fifteen years. Vet. Med., xix (July 1924), pp. 417-418.

[3] Kalantarjan, E. V.: Utilization du nitrate de sodium dans la pratique helminthologique. Med. Parasitol. & Parasit. Dis., vii (1938), pp. 142-143. (Abst. in Helminth. Abst. (1938), vii; part 3—No. 222d.)

(2 lbs., 1 oz.) of zinc sulfate ($ZnSO_4 .7H_2O$, C.P. cryst.) in one liter of distilled water at room temperature, giving a specific gravity of 1.380.

A 62 per cent solution of magnesium sulfate was prepared as recommended by Skriabine and Schulz[4] by dissolving 920 Gm. (1 lb., 15 oz.) of magnesium sulfate ($MgSO_4 .7H_2O$, C.P.) in one liter of distilled water at room temperature. The specific gravity was not given in their report, but by repeated tests with a hydrometer the specific gravity was found to be 1.285.

A saturated sodium chloride solution, as recommended by many workers, contained 360 Gm. (12 oz.) of crystallized sodium chloride in one liter of distilled water, at room temperature. A few grams of salt was added to form an excess in the bottom of the container. This solution had a specific gravity of 1.200.

The sugar solution, first recommended by Sheather,[5] was prepared by dissolving 500 Gm. (1 lb.) of cane sugar in 360 cc. (12 oz.) of distilled water. The water was heated to the boiling point, poured into a receptacle containing the sugar and stirred. To this solution was added 1 per cent (6.5 cc.) of melted phenol crystals as a preservative. The specific gravity was 1.275.

Technic of Fecal Examination.—Fresh fecal material from dogs was collected in paraffined paper cups. Twenty Gm. of this fecal material was placed in a beaker, 80 cc. of distilled water added and a homogeneous suspension formed by thoroughly agitating the feces with a glass stirring rod. This suspension was poured through gauze to remove heavy particles. The resulting suspension contained 1 Gm. of feces in every 5 cc. of suspension.

Flat-bottom vials used in this technic were 5½ cm. high, having a bore of 2½ cm. and holding 21 cc. of fluid. These were ground flat on the top so that the microslide (1″ x 3″) fitted snugly. A circular area, the same as the opening of the vial, was heavily marked on each slide with a soft wax pencil.

Five cc. of the suspension, containing 1 Gm. of feces, was poured into the vial; about 10 cc. of the flotation solution was added and the whole thoroughly mixed with a wooden applicator. Then more solution was added so that the mixture formed a small convex meniscus above the edge of the vial. A prepared microslide was placed on the top of the vial with the wax-marked area resting on the edge and the mixture in the vial touching the surface within the marked circular area.

Two flotation tests were made with each solution at intervals of five and ten minutes. After the elapsed time, the slide was quickly lifted and inverted, so that the surface layer containing the ova was removed and remained inside the wax-marked area. The slide was then examined under the microscope: a mechanical stage was used so that all parts of the wax-marked area would be observed. The ova were counted as they came in view in the field.

The direct centrifugal flotation method was used, as a control measure, with each solution to be tested and with each sample of fecal material. A portion of the same homogeneous suspension (5 cc.) as used in the direct flotation methods was poured into a centrifuge tube (18 cc.) and the solution to be tested was added. The mixture was thoroughly stirred in the tube and then placed in the centrifuge. Enough solution was added to fill the tube slightly above the rim, but not enough to cause overflowing when the cover slip was added. A cover slip 18 mm. square was placed on the top of the tube, which was centrifuged for two minutes. After this the cover slip was lifted from the tube, placed on a 1″ x 3″ slide and the number of ova counted.

The tops of the centrifuge tubes were smoothly ground on a carborundum stone so that the cover slip would fit tightly around the rim and stay on the tube during centrifuging.

RESULTS

Fecal specimens were examined from 45 dogs; 35 specimens contained parasite ova and ten were negative. Nine types of parasite infection, including two species of protozoa, were found in the positive specimens. Some of the specimens contained ova and oöcysts of four different species of parasites, such as *Trichuris vulpis, Taenia pisiformis, Ancylostoma caninum,* and coccidia; one specimen had *T. vulpis, Troglotrema salmincola, Dipylidium caninum,* and coccidia. Many of the other samples contained two or three different types of parasite ova.

Tables I, II and III show the results of a comparison of various flotation solutions in the detection of parasite ova in feces from 45 dogs. *T. vulpis* ova were the most common, being present in 22 of the positive cases. These ova (table I) were found to rise best in the sodium nitrate solution after a five-minute flotation period. An average of 63 ova per gram of feces was

[4]Skriabine, K. I., and Schulz, E. E. S.: Les helminthoses pulmonaries des animaux. Bull. Off. Internat. Epiz., xii (1936), pp. 407-456. (Abst. in Vet. Bul., vii (July 1937), pp. 337.)

[5]Sheather, A. L.: The detection of worm eggs in the faeces of animals, and some experiments in the treatment of parasitic gastritis in cattle. J. Comp. Path. & Therap., xxxvi (June 1923), pp. 71-90.

TABLE I—Comparison of Various Flotation Solutions in the Detection of Parasite Ova in the Feces of Dogs

Name of Parasite	Number Positive	Average Number of Ova per Gram of Feces Counted After an Interval of Five Minutes				
		$NaNO_3$	$ZnSO_4$	$MgSO_4$	NaCl	Sugar
		Specific Gravity				
		1.400	1.380	1.285	1.200	1.275
Trichuris vulpis	22*	63	6	3	0.5	5
Toxocara canis	6	135	107	21	93	59
Toxascaris leonina	3	246	220	69	160	69
Ancylostoma caninum	8	218	43	23	88	29
Troglotrema salmincola	3	573	41	29	0	24
Dipylidium caninum	7	12	1	4	1	0
Taenia pisiformis	2	24	10	0	0	2
Isospora bigemina (oöcyst)	7	200	100	10	100	20
Isospora rivolta (oöcyst)	5	60	8	5	15	14

TABLE II—Comparison of Various Flotation Solutions in the Detection of Parasite Ova in the Feces of Dogs

Name of Parasite	Number Positive	Average Number of Ova per Gram of Feces Counted After an Interval of Ten Minutes				
		$NaNO_3$	$ZnSO_4$	$MgSO_4$	NaCl	Sugar
		Specific Gravity				
		1.400	1.380	1.285	1.200	1.275
Trichuris vulpis	22*	101	8	3	0.7	9
Toxocara canis	6	189	66	26	148	98
Toxascaris leonina	3	464	352	97	266	113
Ancylostoma caninum	8	419	45	33	186	53
Troglotrema salmincola	3	826	116	40	0	36
Dipylidium caninum	7	35	0	9	0	2
Taenia pisiformis	2	35	15	1	0	1
Isospora bigemina (oöcyst)	7	300	200	10	200	104
Isospora rivolta (oöcyst)	5	100	15	10	25	20

TABLE III—Comparison of Various Flotation Solutions in the Detection of Parasite Ova in the Feces of Dogs

Name of Parasite	Number Positive	Average Number of Ova per Gram of Feces Counted After Centrifuging for Two Minutes				
		$NaNO_3$	$ZnSO_4$	$MgSO_4$	NaCl	Sugar
		Specific Gravity				
		1.400	1.380	1.285	1.200	1.275
Trichuris vulpis	22*	248	50	31	0.4	149
Toxocara canis	6	1036	782	464	391	956
Toxascaris leonina	3	948	794	438	424	774
Ancylostoma caninum	8	1135	441	468	356	533
Troglotrema salmincola	3	4333	4453	4167	0	2367
Dipylidium caninum	7	93	1	7	0	10
Taenia pisiformis	2	210	108	2	2	3
Isospora bigemina (oöcyst)	7	3750	2011	150	1000	500
Isospora rivolta (oöcyst)	5	250	142	153	64	91

*Only 13 of these were tested with zinc sulfate.

counted by this method, which was ten times the number counted in the next best solution. The highest individual count with sodium nitrate was 382 ova per gram of feces and the lowest was 1 ovum per gram. All other solutions had some individual negative counts: sugar solution, six negative (27%); zinc sulfate, four negative (31%); magnesium sulfate, eleven negative (50%); and sodium chloride, 18 negative (82%).

With the direct flotation method after an interval of ten minutes, the sodium nitrate solution again had the high average of 101 Trichuris ova per gram, or more than eleven times the number counted in the next best solution. There were no negative results with sodium nitrate in the individual counts, but all other solutions showed some negative counts: sugar, five negative (23%); zinc sulfate, three negative (23%); magnesium sulfate, eleven negative (50%); sodium chloride, 19 negative (86%).

In controlling the experiment a direct centrifugal flotation method was used which, according to Lane,[6] has an accuracy of 80 per cent on the first spin. Sodium nitrate gave an average of 248 ova per gram of feces with no negative individual counts in the 22 positive specimens. The other solutions which gave some individual negative counts were: sugar, four negative (18%); magnesium sulfate, three negative (14%); sodium chloride, 18 negative (82%).

The sodium nitrate, in the 22 positive specimens with *T. vulpis* ova, was far superior in all flotation tests to the other solutions. Even with a centrifuge, the sodium chloride solution was not able to float these heavy ova. In order that we could be certain that ova were present in samples reported negative, the contents of these tubes were mixed with a portion of sodium nitrate solution and examined. In all negative specimens, ova were found by both the direct flotation method and the direct centrifugal flotation method, thereby showing that the specific gravity of these negative solutions was not high enough to float this type of ova.

Six fecal specimens contained *Toxocara canis* ova, and three specimens contained *Toxascaris leonina* ova (tables I, II and III). Again the sodium nitrate in all three flotation tests was superior to the other solutions, while magnesium sulfate gave the poorest results.

A. caninum ova were found in eight specimens. In all three tests (tables I, II and III) the sodium nitrate was vastly superior to the other solutions, having more than twice the number of ova per gram of feces as the next best solution. In the five- and ten-minute flotation tests, there were no negative counts with sodium nitrate, but in one specimen where sodium nitrate floated only two ova in five minutes and three ova in ten minutes, the other solutions were negative.

One series of tests was made from a dog fed salmon infected with *Troglotrema salmincola*. Three specimens of feces, taken after the animal showed symptoms of salmon poisoning, contained many *Tr. salmincola* ova. The best solution (table I) in the five-minute direct flotation test was sodium nitrate, which gave an average of 573 ova per gram of feces, or eleven times as many as the next highest solution. In the ten-minute flotation (table II) sodium nitrate again was superior, with an average of 826 ova, or about eight times as many as the next highest solution. With the centrifuge sodium nitrate, zinc sulfate and magnesium sulfate gave approximately the same average counts. While the counts with other solutions in the five- and ten-minute flotation tests were low, the sodium chloride solution failed to float any ova. In order to check the negative results found with sodium chloride, the contents of these negative tubes were divided and mixed with sodium nitrate and zinc sulfate solutions and tested both by the direct flotation and direct centrifugal flotation method. In all check counts, many ova were found, showing that the Troglotrema ova are too heavy to be floated in sodium chloride solution. With the centrifuge many of the ova were

[6]Lane, C.: The mass diagnosis of hookworm infection. Amer. J. Hyg., viii (May supplement 1928), pp. 1-148.

crushed in the sodium nitrate and zinc sulfate solutions, thus making an accurate count very difficult.

In discussing the specimens containing *D. caninum* ova, one must take into consideration that these ova are not normally released in the intestinal tract but are eliminated in mature proglottides. In one specimen numerous proglottides were passed with the feces, and these were crushed in the suspension. Sodium nitrate gave the best results in all three tests.

The ova of *T. pisiformis*, while normally eliminated with the proglottides as *D. canimum*, are occasionally found in the fecal material. In the two specimens examined that contained these ova, the best results were obtained with the sodium nitrate. Tests with magnesium sulfate and sodium chloride solutions in five-minute flotation and with sodium chloride in the ten-minute flotation were negative.

Two types of oöcysts were found in the feces examined: Seven specimens contained *Isospora bigemina* and five specimens, *I. rivolta*. Sodium nitrate brought more oöcysts to the surface than any other solution, but all gave good results, except magnesium sulfate.

Discussion

Sodium nitrate solution gave a higher average and individual count of ova per gram of feces than any of the other solutions. The ten-minute direct flotation method gave approximately twice the number of ova as did the five-minute direct flotation method. This method was found to be a vast improvement as a diagnostic agent over any other. With the direct centrifugal flotation method there was a marked superiority in ova count over that of either the five- or ten-minute flotation methods. While in some specimens almost ten times as many ova were counted in the centrifugal flotation test as in the direct flotation test, the usual count was three to five times as many.

Zinc sulfate and magnesium sulfate solutions in comparison with sodium nitrate showed inferior results in the direct flotation method. With these solutions the ten-minute test showed only slightly more ova than the five-minute test and in several specimens showed a lower count. More satisfactory results were obtained with the direct centrifugal flotation method, in which zinc sulfate, magnesium sulfate, and sugar solutions were used. In these solutions approximately the same number of ova were floated as in the sodium nitrate (except the ova of Trichuris) and in a few specimens showed a slightly higher ova count.

Ova that have a low specific gravity, such as those of the hookworm, were floated well with sodium chloride solutions in all tests, but with the heavier ova this solution gave inferior results. This was especially the case with the heavy Trichuris ova where many negative counts were obtained, and with *Tr. salmincola* ova where all specimens were negative, even after a direct centrifugal flotation method was used. To check these negative counts, where ova were found with other solutions, the material from the negative count was divided and mixed, one part with sodium nitrate solution and one part with zinc sulfate solution, and centrifuged. In all these checks numerous ova were found.

Even though sodium nitrate solution proved to be superior in the number of ova floated, some faults were found. In some specimens there were numerous bubbles present, making it impossible to count the ova. This was partly overcome by handling the solution carefully and, when mixing with the fecal suspension, pouring carefully and not stirring too roughly. In the slides where bubbles were numerous, a wooden applicator was used to spread the material gently. This removed enough bubbles to make it possible for us to see and count ova, but it was still unsatisfactory. In some specimens where bubbles persisted, sodium nitrate solution was mixed directly with fecal material, and it was found that there were fewer or no bubbles formed in this manner.

Another fault was that considerable debris was floated with sodium nitrate solu-

tion, especially with the centrifuge, making it difficult to see the ova. This was partly overcome by spreading the material over the entire slide in order to make a larger area in which the ova could be seen. In the five- and ten-minute direct flotation method this was not as pronounced as in the direct centrifugal flotation method, in which more debris was floated with sodium nitrate than with any of the other solutions. This fault gave the zinc sulfate, magnesium sulfate, and sugar solutions a better diagnostic value with the centrifuge because the ova were not obscured by debris and bubbles.

Many of the thin-shelled ova were crushed, especially with the direct centrifugal method, making ova counts and even recognition difficult. More ova were crushed with the zinc sulfate solution than any of the other solutions. Sugar solution caused few crushed ova, debris or bubbles. In the direct five- and ten-minute flotation method, in which poor counts were made with zinc sulfate and magnesium sulfate solutions, many crushed ova were seen.

It also was observed that, unless the fecal material and solutions are mixed thoroughly, few ova will appear. With sodium nitrate this was noted several times where few ova appeared, although a large number were expected. In one specimen in which only 70 ova were counted before stirring, 510 ova per gram were counted after stirring; in another specimen in which 72 ova were counted before stirring, 478 ova were counted after stirring. With sodium nitrate solution the stirring or mixing process should be thorough but gentle so as not to cause the formation of bubbles.

With sugar solution thorough mixing is necessary for good results. Several samples that failed to show ova, where many were known to be present, were again rechecked after the same material was mixed thoroughly. In all samples rechecked, where no ova were found before stirring, hundreds of ova were found after stirring.

Successful Chemotherapy of a Virus Disease of the Canary*

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ALTHOUGH virus diseases are widespread in nature, there are few for which there is an effective means of treatment. Indeed, McKinley, Meck and Acree[1] in a recent review of the subject reached this discouraging conclusion: "Our work would indicate that the possibilities of successful chemotherapy in the virus diseases are not particularly encouraging at present." It is true, of course, that means of prevention have been developed for some such maladies, both in man and animals, but the list of those which can not be effectively prevented is still long. Canary pox belongs in this second category and is often the cause of severe losses to breeders and those who use canaries for experimental purposes, as for the study of avian malaria. Our own loss from this cause over a period of ten years has amounted to several hundred birds, and we have been in correspondence with large breeders whose losses have totaled much more than this, sometimes in a single epizoötic. For this reason we became interested in studying the disease and developing, if possible, an effective method of treatment.

MATERIALS AND METHODS

Canary pox is a variety of bird pox, which apparently affects many species of birds, although there are many strains of the virus which, even in the same host, may differ considerably. We have used two strains in the present study: One is of unknown origin, although it may have originated from wild birds which were being brought into the laboratory quite regularly in connection with a study which was being carried out of their parasites. The second strain was secured from a local breeder in whose aviary it had caused severe losses.

Experimental work included (1) efforts to transmit the disease from bird to bird, (2) a study of the pathology and filtrability of the virus, (3) a study of the effect of desiccation on virulence of the virus, and (4) the trial of mercurochrome as a therapeutic agent for treating the disease.

Transmission was attempted by direct contact of infected mucous surfaces, subcutaneous injection of infected tissues, and also by dropping suspensions containing the virus directly into the eyes of healthy birds. Although the disease spreads rapidly in an aviary once it is started, the actual route of spread is not definitely known. In nature mosquitoes (Culex, Aedes, Theobaldia, Anopheles), houseflies, *Stomoxys calcitrans*, certain bugs (*e.g.*, the common bedbug), and certain ticks are said by Findlay[2] to spread fowlpox. Natural transmission of canary pox in our own laboratory could not have occurred in any of these ways, however, since the birds were kept in a screened room and none of the insects or other arthropods on the above list was present. Mites (*Dermanyssus gallinae*) may have played some part, but whatever the usual method of spread may have been, there were often cases in which uninfected and infected birds were kept in the same cages for some time without transmission of the disease, or when it was contracted only after some weeks.

Mercurochrome was used in alcohol-acetone solution (1 to 3 per cent of mercurochrome in 70 per cent alcohol, to which a trace of acetone had been added) and applied once or twice daily to the infected surface, generally with a cotton swab. When there was pharyngeal involvement, a drop or less also was administered orally. The duration of treatment varied with the severity of the disease.

THE DISEASE

Inasmuch as accounts of canary pox differ, and few studies of the disease have been made, it is worth while to give a brief description of it. In our experience it occurs in three forms (although these also may be seen in combination): (1) a form in which the earliest symptom observed is gasping, (2) a second type, in which the chief lesions are found about the margin of the epithelium of the eye, and (3) a third type characterized chiefly by warty outgrowths on the toes and legs. The first

*From the Department of Zoölogy, Syracuse University; this work was aided by a grant from Sigma Xi and the American Philosophical Society.

[1] McKinley, E. B., Meck, J. S., and Acree, E. G.: J. Inf. Dis., lxiv (1939), pp. 36-42.

[2] Findlay, G. M.: Proc. Roy. Soc. Med., xxix (1936), pp. 29-36.

two types have in our experience been uniformly fatal and we have seen at least six distinct epizoötics. The third type has occurred in our laboratory only two or three times and runs a much more chronic course, but after some weeks or months it also kills the bird. Death usually occurs in the first two forms after a week or ten days, and sometimes sooner.

There are many different strains of pox virus, some of them less pathogenic than others, but canary pox as we have seen it behaves as described by other workers in various parts of the world, notably Kikuth and Gollub,[3] Burnet,[4] and Reis and Nobrega.[5] It is clear, therefore, that this highly virulent type is of widespread occurrence. Dividing the disease into types based on symptomatology alone may seem a questionable procedure, but we do it because in some epizoötics one form predominates, and in others another form even almost to the exclusion of the other two.

PATHOLOGY

Both the gross and microscopic pathology were studied, the latter in smears and sections stained with Giemsa. Photomicrographs of the latter are shown in plate I. These exhibit the characteristic hyperplasia of the epithelium produced by the virus of bird pox in such much-studied species as fowl pox, and probably in other hosts as well. Many of the cells show inclusions, such as the Bollinger bodies, and these in turn contain the smaller masses known as Borrel bodies. These bodies are also characteristic of the disease, apparently irrespective of the host species, although the disease itself exists in as many varieties as there are susceptible species of hosts. The epithelial cells are, however, not the only type of cells attacked, at least in the canary, and, according to Burnet,[4] the great pathogenicity of canary pox is due to the fact that macrophages soon become infected and carry the virus throughout the body. Such a monocyte is shown in figure 5. Late in the course of the disease there are marked changes in organs such as the spleen and Burnet states that tissues derived from all three germ layers may be attacked. This fact makes the curative power of mercurochrome, which is discussed below, especially remarkable.

The gross pathology is characterized by initial lesions about the margin of the eye in the ophthalmic type of the disease. These speedily enlarge, and within several days the eye is closed and blindness results on that side. If death does not occur first the disease soon extends to the other side, with similar results.

There are frequently lesions about the beak (fig. 8) and about the nares, particularly in birds which exhibit the gasping type of the disease. Not seldom, however,

[3]Kikuth, W., and Gollub, H.: Zentr. Bact., cxxv (1932), pp. 313-330.

[4]Burnet, F. M.: J. Path. & Bact., xxxvii (1933), pp. 107-122.

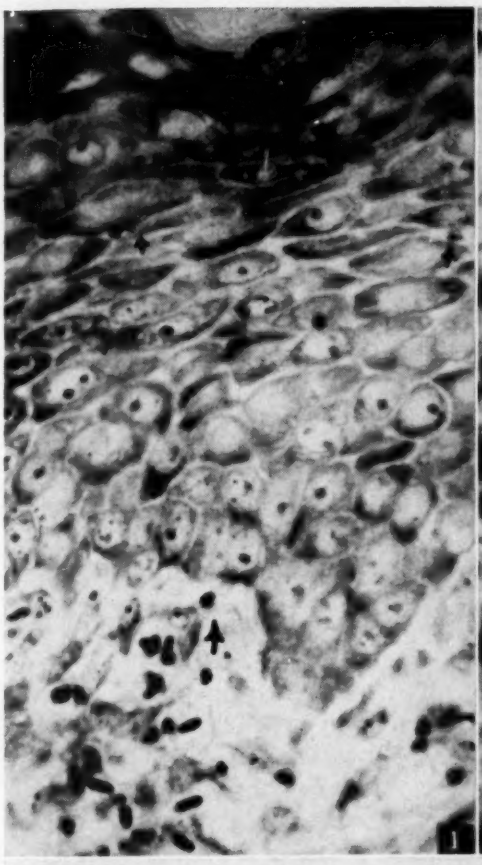
[5]Reis, J., and Nobrega, P.: Arch. Inst. Biol., viii (1937), pp. 211-214.

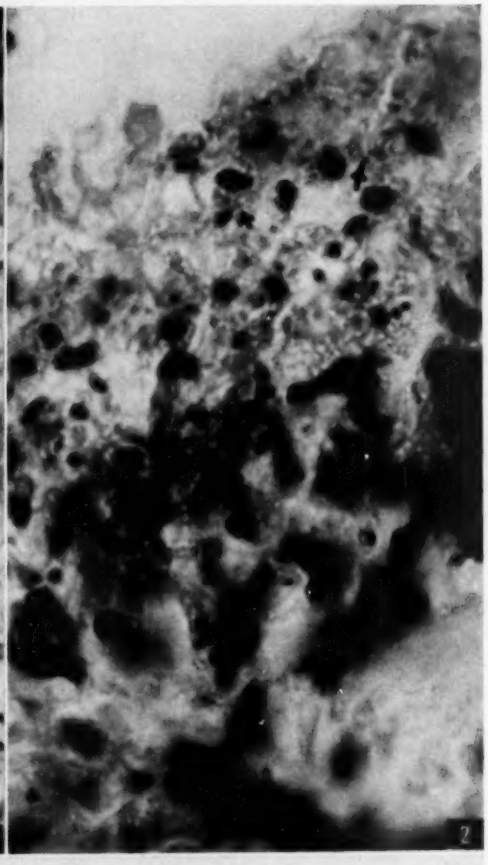
CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

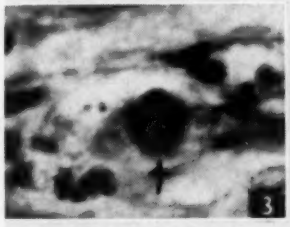
*Plate I—Figs. 1-6**

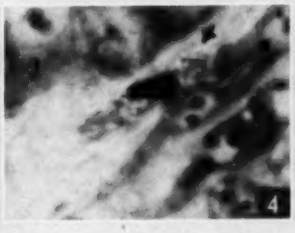
(1) Section through the skin surrounding the eye of an infected bird. The Bollinger bodies are indicated by arrows. In the original preparation these stain bright pink against a blue background (x 750). (2) This section shows marked pathologic changes with many necrotic cells. Bollinger bodies (indicated by arrows) and the Borrel bodies which they contain are so numerous the entire area was stained bright pink (x 1,620). (3) Dark red Bollinger bodies are often seen in the cells of the epidermis, and one of these is indicated (x 1,620). (4) A portion of the epidermis and derma. In the latter a typical Bollinger body containing Borrel bodies is to be seen in a macrophage (arrow). Other inclusion bodies are also visible (x 1,620). (5) From a smear preparation of the epithelioma about an infected eye. A monocyte is shown containing many Borrel bodies (x 1,200). (6) A smear preparation from an eye epithelioma. Borrel bodies are numerous and conspicuous. The Bollinger bodies, which would be evident in a section, appear to be broken in the process of smearing (x 1,200).

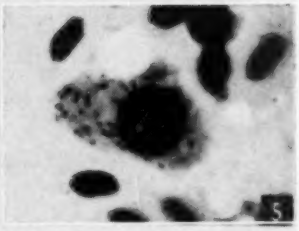
*Photomicrographs from which these illustrations were reproduced were taken by Miss Stella Zimmer, Department of Photography, Syracuse University School of Medicine. Original preparations stained by the Giemsa method.

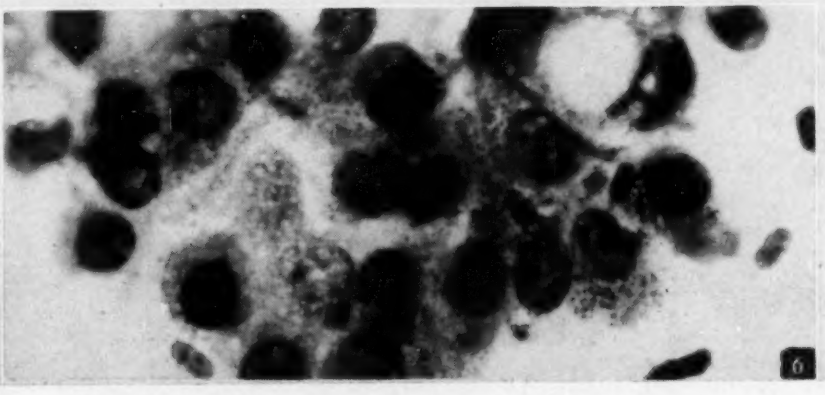












such birds show few and inconspicuous external lesions, but (in our experience) the gasping continues for a week or ten days until the bird dies. It appears that death is directly due to exhaustion, plus inability to take food because of the continuous gasping. But there are also lesions in the pharynx and trachea in these cases, and these are, of course, chiefly responsible for the symptomatology.

TRANSMISSION

Much more is known about the transmission of other varieties of bird pox, particularly fowl pox, than about canary pox. In such host species as the fowl it is said that infection occurs through small abrasions in the skin, or mucous membrane, and by the ingestion of contaminated food. As stated above, the malady also may be spread by biting insects, particularly mosquitoes.

Natural transmission from canary to canary in an aviary is a different matter in many cases. None of the biting insects mentioned above can be incriminated in any of the epizoötics we have experienced, but the mite, *D. gallinae*, as mentioned previously, may have carried infection. Attempts to infect canaries with emulsions of mites taken from cages containing diseased birds have not been successful, however.

The fact that birds which have been kept in cages by themselves frequently develop the disease shows that contamination of food and water, or of parts of the cage, can not always be responsible, and suggests that the virus may be dust-borne.

The predominance of one type of the disease or another in different epizoötics seems to show that the location of the initial lesion is not to be explained simply as the result of original infection at that site. Variation in the virus appears to be a more likely explanation.

Experimentally we have secured infection by direct contact of a lesion with the eye of a clean bird, by subcutaneous and intramuscular injection of a suspension of infected tissue in physiologic saline, by inoculation of blood from a sick bird into a healthy one, and by putting a suspension of the virus into the eye. In all of these cases relatively large doses of the virus were used, but the results suggest that natural infection might also occur, as in other types of bird pox, through abrasions of the skin and mucous surfaces. It is worth noting that the incubation period may be very long, although it is stated by Reis, Nobrega and Reis[6] to be about four to six days on the average. In the case of one bird, which we infected by dropping the virus into the eye, the disease required five weeks to develop.

It is probable that transmission under natural conditions is frequently the result of contact of mucous surfaces with the perches or other parts of the cages. Sick birds are in the habit of almost continually rubbing their beaks, around which there are likely to be active lesions, on the perches and we have found the virus to retain its virulence for a number of months. It is clear that contaminated parts of the

[6]Reis, J., and Nobrega, P., with Reis, A. S.: Tratado de doencas das aves. (Chapt. 2, 1936, pp. 13-30.)

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

Plate II—Figs. 7-15

(7) The eye of an infected bird before treatment was begun. It would require several days after the appearance of the first symptoms for the disease to reach this stage. (8) Same bird as in figure 7, showing a lesion under the beak. The bird was gasping. (9) Appearance of the eye after three days of treatment. (10) One week later. The lesion under the beak has disappeared. New tissue is filling the space occupied by the scab observed in figure 9. (11) Fifteen days after treatment was started. Healing is virtually complete and new feathers have begun to develop where the lesion was. (12) The other eye of the subject, taken at the same time as figure 7. (13) The treated eye three months after treatment was instituted. It is normal, although feathers growing on the site of the former lesion might be taken for a scar. (14) An advanced epithelioma, showing extensive epithelial proliferation characteristic of the disease. This case was treated successfully. (15) In this case lesions about the eye and nare are clearly evident.

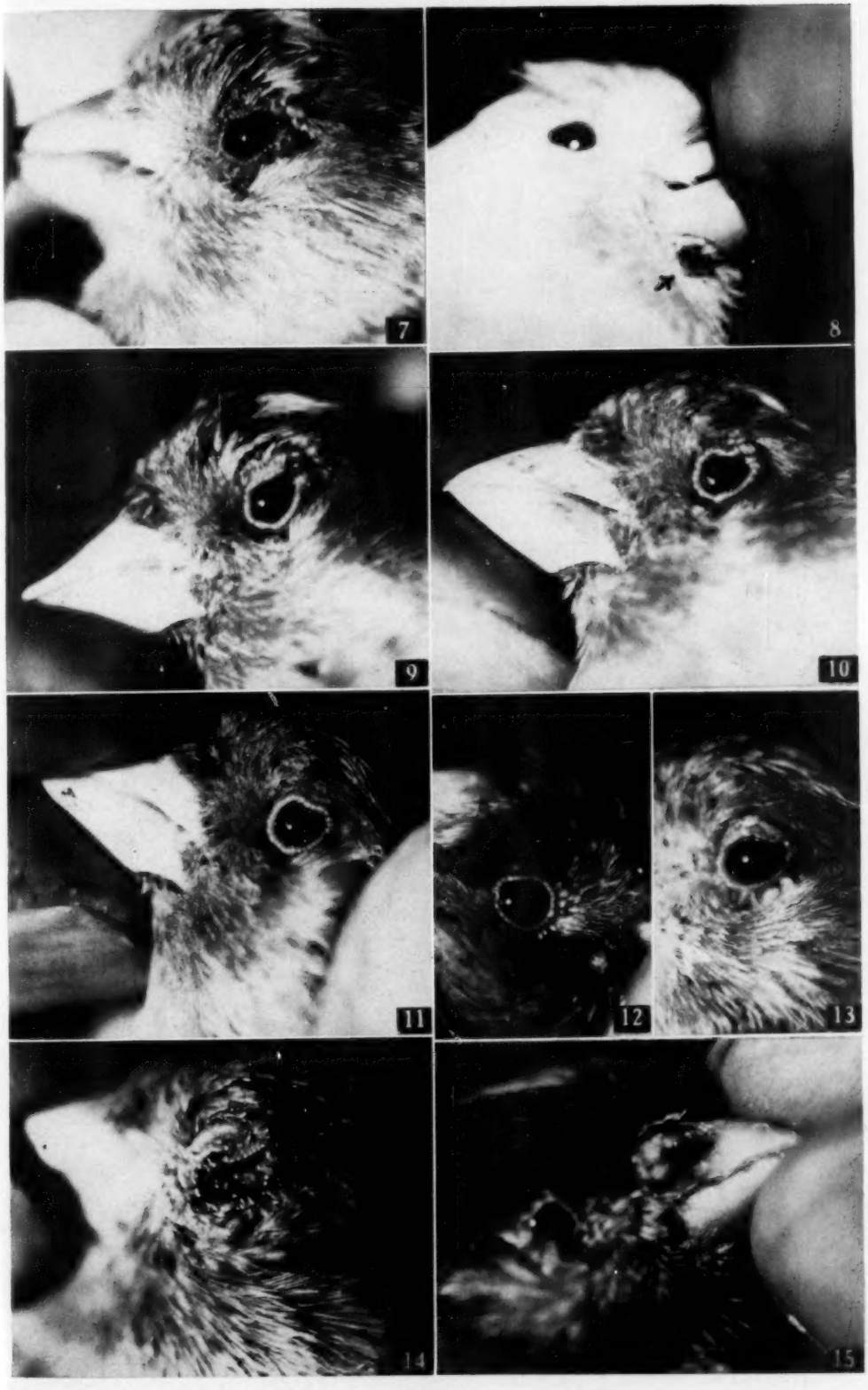

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TABLE I—Summary of Results of Treating Canary Pox with Mercurochrome

Number of Cases	Mode of Infection		Treatment			Effect on Disease				
								Recovery		
	Naturally Acquired	Inoculated*	Stage of Infection†	Number of Applications‡	Duration (Days)	Symptoms	Lesions	Time Required (Days)	Proportion Recovered	Mortality
20	16	4	Early	4–10	5–14	Disappeared	Disappeared	4–9	100%	None
8	6	2	Advanced	8–16	10–26	Disappeared	Disappeared	5–17	100%	None
12	5	7	Very advanced	10–20	15–30	Alleviated slowly	Advanced more slowly	10–24	42% (5 cases)	7 cases (All deaths delayed)

*All injected subcutaneously under eye.

†Early = Early symptoms present. Advanced = Epitheliomas; eye still open. Very advanced = Epitheliomas large; eye completely closed.

‡Mode of treatment: Local and oral with swab and dropper.

cages would, therefore, remain infective for a long time if not sterilized.

TREATMENT

Various methods of treatment have been tried in fowl pox, among them tincture of iodine with glycerin, and protargol. But apparently there has been no successful method of treating canary pox. The effect of mercurochrome in an alcohol-acetone solution, however, is striking, and is illustrated in plate II. We have used it on 40 cases, with the results summarized in table I. Except in very advanced cases, treatment is almost always effective. Untreated birds, on the contrary, apparently always die, whether the method of infection is natural or artificial.

The amount and efficacy of treatment vary with the severity of the case, as might be expected. Not infrequently, when treatment is started early, it appears that one or a very few treatment(s) repeated once or twice daily is/are sufficient. Both the gasping and the ophthalmic type of the disease are susceptible to treatment, but in the former we have given a little mercurochrome orally, as well as applying it to the visible lesions.

The effect of treatment is graphically shown in plate II, in which figures 7 to 13 depicit the same bird before treatment, and after treatment has been continued for varying lengths of time. At times cases exhibit both lesions of the eye and of the mouth parts and upper respiratory passages, as this bird did. Although seriously ill when treatment started, and gasping so much that a good picture was difficult to obtain, it exhibited steady improvement and after two weeks of treatment was virtually well. In this case the drug was administered twice a day for ten days, and once daily every other day until four additional applications had been made. Improvement is evident in such cases almost immediately, and in this one the gasping stopped within 24 hours.

When it is recalled that, in our experience, canary pox has proved uniformly fatal without treatment, the results of mercurochrome administration seem remarkable. Possibly other drugs also would prove effective, but iodine at least seems too harsh a drug for application to the eye or even to give internally unless in minute quantity. As a matter of fact, we have tried several other drugs, including 70 per cent alcohol (since the mercurochrome is dissolved in alcohol), merthiolate (1:1,000) applied locally, and prontylin, but none of these substances has exhibited any effect.

Relapse after recovery seems to be uncommon. We have observed three such cases, but a few more applications of mercurochrome were sufficient to bring about complete and permanent recovery.

The fact that relapses do occasionally occur leads to the question of whether or not recovery is accompanied by immunity to reinfection. It is true that in fowls and in a few other species of hosts which have

...n studied, recovery from the pox leaves ... immunity. It is also true, however, that ...ere are many strains of bird pox, and that these differ much among themselves. ...me are highly pathogenic and others are much less so. Lahaye[7] and Eberbeck and Kayser[8] studied a strain of bird pox which was infective for canaries, but relatively nonpathogenic. It is almost a commonplace that such viruses differ much in their specificity, and we have found the strains of canary pox used in this study incapable of infecting starlings, cowbirds and chickens, but capable of producing a rather mild infection in song sparrows and English sparrows. When differences as great as these exist, it seems dangerous to make *a priori* assumptions for one host species or virus strain on the basis of others. As a matter of fact, six cases in which recovery had been brought about by treatment were reinfected with large doses of virus, and found to be refractory.

The infectivity of the virus after being kept in a desiccated condition for varying lengths of time also was studied, and it was found that after seven or eight months the virulence was considerably diminished, and after eleven months completely lost. Eight cases which had recovered naturally after infection with the virus attenuated in this manner were reinoculated, four with another dose of attenuated virus, and four with virulent virus. The first four proved resistant to reinfection, but the virulent virus produced fatal infections in two of the second group. (In all cases the virus was inoculated subcutaneously under the eye.) Thus it appears that immunity to reinfection is only relative, but it is sufficiently strong after infection with attenuated virus to suggest the possibility of successful vaccination as a preventive.

The possibility that recovered cases may carry the virus for a time, and thus act as carriers, may be mentioned. The fact that relapses do not often occur, and that we have found the blood and organs of such cases noninfectious, and that birds kept in the same cage with recovered cases remain well, seems to make it certain that carriers occur rarely.

[7] Lahaye, J.: Ann. de méd. vét., lxxiv-lxxv (1929-30) p. 515.

[8] Eberbeck, E., and Kayser, W.: Arch. Tierh., lxv (19...2), p. 307.

SUMMARY AND CONCLUSIONS

Canary pox is a virus disease of canaries which often appears in highly virulent form, killing virtually all birds which it attacks. It tends to occur in two forms which are symptomatically different: In the one there are swellings about the eyes and often elsewhere on the head, and in the other the chief indication is continual gasping. The virus nature of the disease is established by the occurrence of typical cellular inclusions, and by successful filtration through a Berkefeld candle ("W"). It has been found infective for English and song sparrows, but not for cowbirds, starlings and chickens. When dried, the virus retains its virulence for about seven months, is capable of producing relatively mild infections for about two months longer, and loses its virulence completely thereafter. Canaries which recover after being infected with the attenuated virus develop an immunity which protects them to a considerable extent, but not completely, against the completely virulent virus, thus suggesting the possibility of successful vaccination. Whenever infection with the virulent stock of virus has been undertaken, however, the result has been fatal, and the site or means of transmission has not appeared to make any difference. The natural method of transmission is not known, but it does not involve any of the species of insects thus far reported as vectors of bird pox.

Treatment of the disease is possible with mercurochrome in an alcohol-acetone solution. The drug is applied both locally and, in very small doses, orally. This method of treatment is successful in nearly all cases, unless they are very advanced. Recovered birds exhibit a strong immunity to reinfection, particularly if they have been infected to begin with by a virulent virus.

Use of Tin Preparations for the Treatment of Chickens Experimentally Infected with Tapeworms*

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EVER SINCE kamala has been shown to be unsatisfactory as a taeniacide for use in poultry, there has been a need for an effective therapeutic agent to be used for expelling tapeworms from fowls. Accordingly, numerous compounds have been tested in the laboratories of the Zoölogical Division of the Bureau of Animal Industry to determine whether or not they would show taeniacidal properties. Of the compounds tested, those containing tin showed more or less promise. Although it is not possible to recommend at present any of the tin preparations which have been tested, the data collected show sufficient promise to warrant their publication.

REVIEW OF LITERATURE

The literature dealing with those substances which have been recommended and used for the treatments of tapeworm-infested poultry has been reviewed in a previous publication[1] and need not be repeated here. However, a brief review of the literature dealing with the therapeutic value of tin follows.

Tin is probably one of the oldest metals known to man. Tin mines in Cornwall are thought to have been worked by the Phoenicians about 1000 B. C. The metal is mentioned in Biblical literature and also by Pliny in his *Historia Naturalis*. As early as 1589 Paracelsus[2] reported that tin filings possessed anthelmintic properties. In 1742, Alston[3] gave for use in man the following formula which, he stated, came to his attention in 1719: "Take an ounce and a half of pewter metal and grind it small to powder; take half a Mutchkin (a Mutchkin equals 0.9 U. S. pint) of Treacle and take your powder and mix both together. The Friday before the change of the moon take one-half of it and the day thereafter take the other half of the other half and the Sunday thereafter the rest of it; on the Monday purge." This medicament was administered with success to a woman who had long been troubled with tapeworms. Richter[4] discussed the therapeutic applications of tin and referred to several investigators who had used this substance as an anthelmintic, chiefly against tapeworms.

The use of tin as a taeniacide apparently declined until 1889, when Dommes[5] reported favorably on its use as an anthelmintic. He used a fine gray electrolytic powder in doses of 7.5 gr. four times daily and stated that no toxic effects were noted. Dochevskii[6] administered tin in daily doses of 0.5 Gm. to two dogs and noted no untoward effects. He stated that he experienced no unpleasant results after taking large doses of tin himself and that it appeared to be relatively nontoxic. Four patients treated with powdered tin, in doses of 1.5 to 3.6 Gm., eliminated complete specimens of *Taenia saginata*. Delektorskii[7] reported on the use of tin as a remedy for tapeworms in children. The patients were kept on a diet of milk, broth and bread for two days, during which time they were purged vigorously. Powdered tin in doses of 1.2 to 5.1 Gm. was administered on the second day. The scolex was expelled in only one case out of a total of ten so treated. Fedorov[8] administered tin in a total of 20 tapeworm cases from which one head was obtained. Lepinay[9] reported that pure oxide of tin administered to dogs removed a large number of tapeworms including the heads. The same treatment removed two specimens of *T. saginata* from a man, but the heads could not be demonstrated. In 1939, *The Journal of the American Medical Association*[10] published a statement that tin is of no value as a taeniacide.

A mixture of metallic tin and tin oxide has been employed to some extent, especially in France, for the treatment of furunculosis and other staphylococcic infections, the treatment having been introduced in 1917 by Frouin and Gregoire.[11] Compton,[12] Wright[13] and Sellier[14] reported favorably on tin therapy in connection with the treatment of skin infections in man and small animals, but Kolmer *et al* [15, 16] were unable to prove experimentally the therapeutic value of tin compounds in staphylococcic infections of rabbits and guinea pigs. Oral tin therapy exhibited definite curative properties in 18 cases of chronic osteomyelitis treated by Klein,[17, 18] as well as in hordeolosis, according to Levy[19] and Schlesinger.[20]

The toxicity of tin has been investigated by several workers. However, the early observations on the poisonous properties of this metal are unreliable, since in most instances the possibility exists that the tin was mixed with

*From the Zoölogical Division, Bureau of Animal Industry, U. S. Department of Agriculture.

arsenic or lead. White[21] reported that 2.5 mg. of triethyltin acetate was fatal to a large frog in eight to ten hours, and the fatal dose of the same compound for a large rabbit was 20 mg. distributed over a period of three days. Intravenous injection of 22 mg. of tin in the form of sodium stannous oxide tartrate was fatal to a medium-size rabbit on the fifth day. This author gives the symptoms of tin poisoning as nausea, vomiting, loss of appetite, thirst, profuse diarrhea and increased reflex irritability followed by paralysis. Ungar and Bödlander[22] also investigated the toxicity of tin in the form of sodium stannous oxide tartrate. They observed that this compound was much less toxic when administered in the feed than when given subcutaneously. Daily doses of .08 Gm. of stannous chloride were apparently well tolerated by a dog weighing 5 kg.

Salant *et al*[23, 24, 25, 26, 27] reported that administration of tin as tin tartrate by the mouth caused vomiting and diarrhea in cats when given in 10-mg. amounts. The same symptoms were exhibited by dogs given 25-mg. doses. Intravenous injection of 20 mg. of tin per kilogram, in the form of sodium stannous tartrate, proved fatal to laboratory animals within 24 hours. Feeding experiments with rats carried on over a period of four to twelve months failed to show any toxic effect, although absorption of the metal took place to some extent. Loss of weight was noted in cats fed tin as tin tartrate and tin chloride, in daily doses of 50 mg. per kilogram for periods as long as four weeks. Schryver[28] took the double tartrate of tin and sodium for three weeks. The daily dose was 1 gr. the first week, 2 gr. the second week and 3 gr. the third week. The amount of tin in the urine and feces was determined daily. During the first two weeks the output kept pace with the intake, but some was retained during the third week. Similar experiments with dogs gave practically the same results. Goss[29] determined that tin is readily adsorbed from solutions by coagulated proteins. This adsorption complex was very stable as both peptic and tryptic digestion were markedly interfered with. On the basis of these findings, the author reasoned that digestion of such compounds would be inhibited to such an extent that tin combined in this way could exert little if any toxic effect. Hanzlik *et al*[30] reported that tin given in amounts 2½ times the fatal dose of lead was not toxic to pigeons during 27 days of observation. Chemical analysis of the organs of normal horses and cattle made by Bertrand and Ciurea[31] showed that tin was present in all organs examined with the exception of the peritoneum.

MATERIALS AND METHODS

All chickens used in the experiments reported in this paper were obtained as 1-day-old chicks from the Animal Husbandry Division of the Bureau of Animal Industry at the Beltsville Research Center, Beltsville, Md. The birds were kept under conditions designed to prevent extraneous infections with helminths, and were fed a well-balanced diet consisting of ground corn, wheat middlings, alfalfa meal, dried milk, meat scraps, fish meal, cod liver oil and salt.

Meal beetles (Tribolium spp.) were fed on gravid segments of *Raillietina cesticillus*. After a period of five to six weeks each beetle was dissected in a small amount of physiologic saline, and the fully developed cysticercoids obtained were counted and administered to each bird by means of a glass pipette. Each bird used for the preliminary testing of the drugs was given twelve cysticercoids. For the controlled tests on the more promising drugs, birds infected with 50 cysticercoids each were employed.

Two to four weeks after infection each bird was confined in separate compartments of a laying battery having a wire floor through which the droppings passed into a pan containing a small amount of water. The drugs were administered in hard gelatin capsules to the birds after a fast of 18 to 24 hours. Each capsule was pushed well down into the esophagus and then on into the crop by grasping the throat region between the thumb and forefinger and forcing the capsule ahead. Four hours after treatment the regular feed was placed before the birds. The feces of each bird were collected daily for three days and examined in the usual manner for tapeworms. Each bird was weighed the day of treatment and on the second, fourth and 14th day following treatment. Two weeks after treatment each bird was killed and the intestinal tract carefully examined for tapeworms. The controlled tests were conducted as described above, except that each bird was given 50 cysticercoids instead of twelve, and an adequate number of control birds, which had been infected with tapeworms, were kept with each group of treated chickens. At the time of treatment the control birds were fasted as described above and given an empty capsule similar to that used for the administration of the drug to the treated birds.

The dung beetle, *Onthophagus hecate*, was used as an intermediate host for *Hymenolepis carioca*. The procedure was similar to that used in the controlled tests with *R. cesticillus*, except that 1,000 cysticercoids were administered to each bird. Because these birds were used in connection with an experiment intended to determine tapeworm injury, it was necessary to necropsy the treated birds two weeks after the date of necropsy of the control birds. Since tapeworm infections of chickens are usually of long duration (Harwood,[32] Ackert[33] and Chandler[34]) this delay is a matter of little or no

TABLE I—Effects of Inorganic Tin Compounds on Experimental Infections of Raillietina Cesticillus in Chickens*

Band No. and Breed†	Weight (Gm.)	Date of Infection (1939)	Drug Employed	Dose (Gm.)	Date of Treatment (1939)	Date of Necropsy (1939)	Tapeworms Recovered at Necropsy (No.)	Effect of Treatment on Host
539 L	1,328	1/6	Stannous chloride	0.2	1/20	2/3	5	None
3511 M	407	1/6	Stannous chloride	0.2	1/24	2/7	7	None
3590 M	484	1/6	Stannous chloride	0.35	1/30	2/13	4	None
3919 RI	1,020	1/23	Stannous chloride	1.1	2/7	2/21	0	None
3551 M	1,087	2/13	Stannous chloride	3.0	3/1	3/10	0	Died
1874 L	1,040	4/22	Stannic iodide	0.8	8/21	9/4	12	None
1983 LS	974	4/11	Stannic iodide	0.5	7/20	8/3	0	None
1852 LS	1,098	4/22	Stannous oxide	3.0	7/20	8/3	3	None
1934 M	440	4/11	Stannous oxide hydrate	0.1	5/15	5/29	1	None
1986 M	530	4/11	Stannous oxide hydrate	0.2	5/15	5/29	0	None
1984 M	335	4/11	Stannous oxide hydrate	0.3	5/15	5/29	0	None
1997 M	425	4/11	Stannous oxide hydrate	0.4	5/15	5/29	1	None
1911 M	415	4/11	Stannous oxide hydrate	0.5	5/15	5/29	1	None
1995 M	398	4/11	Stannous oxide hydrate	0.5	5/9	5/23	1	Lost weight
1996 M	370	4/11	Stannous oxide hydrate	0.7	5/15	5/18	0	Died
3592 M	486	1/6	Stannous oxide hydrate	0.8	1/30	2/14	1	None
1922 M	460	4/11	Stannous oxide hydrate	0.9	5/15	5/29	0	Lost weight
1981 M	445	4/11	Stannous oxide hydrate	1.0	5/9	5/23	8	Toxic
1993 M	545	4/11	Stannous oxide hydrate	1.5	5/9	5/23	0	None
1954 M	352	4/11	Stannous oxide hydrate	1.5	5/4	5/18	7	Toxic
244 RI	920	3/22	Stannous oxide hydrate	5.0	4/24	5/8	0	Lost weight
1784 LS	1,040	4/22	Stannous phosphate	2.0	7/20	8/3	1	None
1794 LS	956	4/22	Stannous sulfate	1.0	7/20	8/3	5	Lost weight
1736 LS	920	4/22	Stannous sulfide	2.0	7/20	8/3	3	None

*These birds were infected with twelve cysticercoids each.

†Breeds are indicated by letters following the band number: L—Leghorn, M—Mixed, RI—Rhode Island Red, LS—Light Sussex.

consequence, as far as the experiments discussed in this paper are concerned.

DATA

Table I presents a summary of the results obtained by the administration of seven inorganic tin compounds to chickens experimentally infected with twelve cysticercoids each of *R. cesticillus*. The most encouraging results were obtained from the administration of various amounts of stannous oxide hydrate prepared in the laboratory by the authors of this paper. The other compounds were not tested as extensively, but from the observations made at the time of treatment and the data presented, they appear to be less effective.

The results obtained from the administration of five organic tin compounds to chickens experimentally infected with twelve cysticercoids each of *R. cesticillus* are recorded in table II. Tin oleate,* stannous oxalate and stannous tartrate were of low toxicity and on the basis of the data presented, they are the most promising for anthelmintic use. Triphenyl tin chloride was found to be too toxic to warrant further testing. On the other hand, tetraphenyl tin, while of low toxicity, was ineffective in all dosages used.

By mixing various tin compounds with a small amount of synthetic pelletierine hydrochloride, the taeniacidal activity of these compounds was greatly enhanced. The results obtained from the administration of these and of other mixtures of less promise to birds infected with twelve cysticercoids each of *R. cesticillus* are reported in table III. Mixtures containing one of the following tin salts, namely, stannous oxalate, stannous oxide hydrate or stannous tartrate, as well as a small amount of synthetic pelletierine hydrochloride, exhibited most encouraging taeniacidal properties. The stannous chloride-pelletierine mixture proved to be somewhat toxic and further work with it was discontinued.

Since preliminary tests with various tin compounds, and mixtures containing tin compounds and synthetic pelletierine hydrochloride, were promising on chickens infected with a small number of tapeworms,

*The sample of "tin oleate" used was obtained from commercial sources, and nothing is known of its purity or chemical composition other than the name implies. However, two samples of "stannous oleate" obtained from another source appeared to be ineffective. As the "tin oleate" and the "stannous oleates" differed markedly in physical appearance, it is probable that these materials from different sources are of different chemical composition. The problem is being investigated at the present time.

it was deemed necessary to obtain more complete information on the efficacy of these substances by using controlled tests. The results obtained in these controlled tests are reported in table IV. As shown in the table, mixtures containing a small amount of synthetic pelletierine hydrochloride and one of the following tin compounds, namely, tin oleate, stannous oxalate, stannous oxide hydrate or stannous tartrate, removed *R. cesticillus* from heavily parasitized birds with a high degree of efficacy. Examination of the feces after treatment with these mixtures revealed the presence of numerous strobilae, but in only a few instances were the heads recovered. It is possible that the active principle of these mixtures causes the strobila to break off near to the scolex which remains attached and later is destroyed, or that the drug hastens the disintegration of the heads to such an extent that they are digested in the intestinal tract.

These tin pelletierine mixtures were apparently somewhat irritating to the intestinal tract, as shown by the presence of some mucus in the feces the day following treatment. However, the treatment did not seriously affect the health of the birds, since the gain in weight of the treated birds compared favorably with that of the controls in nearly every case. In some instances the treated birds actually gained more weight than the controls, but until more data are available no significance can be attached to this observation.

During the course of these investigations 18 birds infected experimentally with 1,000 cysticercoids each of *H. carioca* became available. As has already been explained, it was necessary to necropsy the controls two weeks before the treated birds. Since more work had been done at that time with the stannous tartrate-pelletierine mixture, this combination was chosen for the tests with these birds. The results obtained, as reported in table V, indicate that this mixture is even more effective for the removal of *H. carioca* than for *R. cesticillus*.

Since the tin soap-pelletierine mixtures proved to be effective taeniacides in these tests, it became necessary to determine the toxicity of these substances when administered to chickens in large doses. Accordingly, several experiments were performed in which the drugs were administered after

TABLE II—Effects of Organic Tin Compounds on Experimental Infections of Raillietina Cesticillus in Chickens*

Band No. and Breed†	Weight (Gm.)	Date of Infection (1939)	Drug Employed	Dose (Gm.)	Date of Treatment (1939)	Date of Necropsy (1939)	Tapeworms Recovered at Necropsy (No.)	Effect of Treatment on Host
296 RI	735	3/3	Tetraphenyltin	5.0	3/28	4/11	3	None
3508 M	1,118	2/13	Tetraphenyltin	1.0	3/20	4/11	5	None
1860 LS	1,010	4/22	Triphenyltin chloride	0.5	7/20	7/24	0	Died
1974 M	490	4/11	Triphenyltin chloride	1.0	5/23	5/24	0	Died
177 RI	1,055	3/22	Stannous tartrate	1.0	4/10	4/24	10	None
3587 M	1,020	2/13	Stannous tartrate	1.0	3/20	4/4	0	None
229 RI	1,065	3/22	Stannous tartrate	2.0	4/10	4/24	5	Lost weight
221 RI	1,140	3/22	Stannous tartrate	3.0	4/10	4/24	5	Lost weight
1904 M	410	4/11	Tin oleate	0.5	5/23	6/6	0	None
1727 M	395	4/22	Tin oleate	1.0	5/23	6/6	0	None
1917 M	385	4/11	Tin oleate	1.5	5/23	6/6	2	None
1215 RI	645	9/13	Tin oleate	2.0	10/4	10/6	0	Died
3549 M	1,105	2/13	Tin oleate	2.0	3/20	4/4	0	Lost weight
1932 M	371	4/11	Tin oleate	2.0	5/23	6/6	0	None
233 RI	988	3/3	Tin oleate	2.0	4/10	4/24	2	None
1706 M	410	4/23	Tin oleate	2.5	5/23	6/6	4	None
201 RI	1,115	3/22	Tin oleate	3.0	4/17	5/1	0	None
155 RI	825	3/22	Tin oleate	3.0	4/10	4/24	4	Lost weight
1714 M	450	4/23	Tin oleate	3.0	5/23	6/6	0	None
1908 I	340	4/11	Tin oleate	3.2	4/28	5/12	0	Toxic
1733 L	1,045	4/22	Stannous oxalate	0.5	8/21	9/4	7	Lost weight
1855 LS	809	4/22 (1940)	Stannous oxalate	1.0	7/20 (1940)	8/3 (1940)	0	Lost weight
1553 RI	645	1/12	Stannous oxalate	0.2	2/12	2/26	1	Lost weight
1550 RI	435	1/12	Stannous oxalate	0.4	2/12	2/26	0	Lost weight
1523 RI	630	1/12	Stannous oxalate	0.6	2/12	2/26	3	None
1544 RI	670	1/12	Stannous oxalate	0.8	2/12	2/26	1	Lost weight
1537 RI	615	1/12	Stannous oxalate	1.0	2/12	2/26	0	Lost weight

*These birds were infected with twelve cysticercoids each.

†Breeds are indicated by letters following the band number: L—Leghorn, M—Mixed, RI—Rhode Island Red, LS—Light Sussex.

TABLE III—Effects of Various Mixtures Containing Tin Compounds on Experimental Infections of Raillietina Cesticillus in Chickens*

Band No. and Breed†	Weight (Gm.)	Date of Infection (1939)	Drug Employed	Dose (Gm.)	Date of Treatment (1939)	Date of Necropsy (1939)	Tapeworms Recovered at Necropsy (No.)	Effect of Treatment on Host
239 RI	735	3/3	Stannous chloride	1.0	3/28	4/11	1	Lost weight
			Aluminum hydroxide	2.0				
1941 M	390	4/11	Stannous chloride	0.25	5/4	5/5	0	Died
			Synthetic pelletierine HCl	0.125				
931 M	365	4/11	Stannous chloride	0.1	5/9	5/23	0	Toxic
			Synthetic pelletierine HCl	0.1				
1972 M	400	4/11	Stannous chloride	0.2	5/9	5/23	0	Lost weight
			Synthetic pelletierine HCl	0.15				
1725 L	925	4/22	Stannous chloride	0.3	8/21	9/4	0	None
			Synthetic pelletierine HCl	0.15				
128 RI	675	3/3	Stannous oxide hydrate	2.0	3/28	4/11	0	None
			Aluminum hydroxide	2.0				
275 RI	713	3/22	Stannous oxide hydrate	0.5	4/10	4/24	1	None
			Synthetic pelletierine HCl	0.125				
163 RI	880	3/22	Stannous oxide hydrate	1.0	4/10	4/24	2	None
			Synthetic pelletierine HCl	0.1				
1714 L	1,029	4/22	Stannous oxide hydrate	1.0	8/21	9/4	2	None
			Synthetic pelletierine HCl	0.15				
1949 M	400	4/11	Stannous oxide hydrate	1.0	5/4	5/18	0	None
			Synthetic pelletierine HCl	0.25				
175 RI	990	3/22	Stannous oxide hydrate	1.0	4/10	4/24	0	None
			Synthetic pelletierine HCl	0.25				
138 RI	1,360	3/22	Stannous oxide hydrate	2.0	4/24	5/8	0	Lost weight
			Synthetic pelletierine HCl	0.5				
383 M	393	3/11	Tin oleate	2.0	5/4	5/18	0	None
			Aluminum hydroxide	1.0				
1948 L	745	4/11	Tin oleate	2.0	8/31	9/13	0	Lost weight
			Calcium arsenate	0.2				
3815 M	420	4/11	Stannous tartrate	0.8	5/4	5/18	3	Toxic
			Aluminum chloride	1.0				
189 RI	655	3/3	Stannous tartrate	3.0	3/28	4/11	5	Lost weight
			Aluminum hydroxide	2.0				
1938 M	455	4/11	Stannous tartrate	0.25	5/23	6/6	0	None
			Synthetic pelletierine HCl	0.1				
1926 M	358	4/11	Stannous tartrate	0.5	4/28	5/12	0	None
			Synthetic pelletierine HCl	0.2				
1935 M	470	4/11	Stannous tartrate	0.5	5/23	6/6	0	Lost weight
			Synthetic pelletierine HCl	0.1				
1996 M	550	4/11	Stannous tartrate	0.5	5/23	6/6	0	None
			Synthetic pelletierine HCl	0.2				
1967 M	1,065	4/11	Stannous tartrate	1.0	8/14	8/28	0	None
			Synthetic pelletierine HCl	0.2				
292 RI	1,095	3/22	Stannous tartrate	2.0	4/24	5/8	0	Lost weight
			Synthetic pelletierine HCl	0.5				
		(1940)			(1940)	(1940)		
1546 RI	520	1/12	Stannous oxalate	0.2	2/12	2/26	0	None
			Synthetic pelletierine HCl	0.1				
1542 RI	575	1/12	Stannous oxalate	0.2	2/12	2/26	0	None
			Synthetic pelletierine HCl	0.2				
1552 RI	580	1/12	Stannous oxalate	0.5	2/12	2/26	0	Lost weight
			Synthetic pelletierine HCl	0.2				
1547 RI	602	1/12	Stannous oxalate	0.3	2/12	2/26	4	Lost weight
			Synthetic pelletierine HCl	0.15				

*These birds were infected with twelve cysticercoids each.
†Breeds are indicated by letters following the band number: L—Leghorn, M—Mixed, RI—Rhode Island Red.

a fasting period of 18 hours. The details of these experiments appear in table VI.

A comparison of the data relating to toxicity of the various medicaments and those relating to their efficacy suggest that the mixture of tin oleate with synthetic pelletierine hydrochloride is the most promising preparation tested. On the basis of the limited data available it is impossible to form a definite opinion as to the therapeutic index of this mixture, but it appears to be as high as 1:5, and it may possibly be as high as 1:10. Until data concerning the efficacy of this mixture for the removal of cestodes other than *R. cesticillus* from chickens are available, more accurate information on the therapeutic index is deemed unnecessary. From table VI it may be seen that two chickens (1197, 1132) withstood a dose of 10 Gm. of tin oleate without deleterious effects. However, 10 Gm. of tin oleate combined with 1 Gm. of synthetic pelletierine hydrochloride was fatal to two other chickens. It is probable, therefore, that the synthetic pelletierine hydrochloride contributed to the toxicity of the mixture.

Discussion

It is evident from the data presented in tables I and II that inorganic and organic tin compounds are of slight value for the

TABLE IV—Efficacy of Tin Compounds and of Tin Compounds Plus Synthetic Pelletierine Hydrochloride for the Removal of Raillietina Cesticillus from Experimentally Infected Chickens*

Experiment No.	No. of Birds in Experiment	Date of Infection (1939)	Drugs Employed	Dose (Gm.)	Date of Treatment (1939)	Mean Weight at Time of Treatment (Gm.)	Mean Gain in Weight During 4 Days Following Treatment (Gm.)	Tapeworms Recovered at Necropsy† (Mean No.)	Efficacy of Treatment (Per Cent)
I	10	5/17 18	Tin oleate	2.5	6/5	399.7	46.6	4.5	83.2
	5	5/17 18	Controls			405.2	86.7	26.8	
II	10	5/17 18	Stannous oxide hydrate	1.0	6/12	422.8	40.6	10.2	57.85
	10	5/17 18	Stannous tartrate Pelletierine HCl	0.3 0.07	6/12	426.9	66.2	6.2	74.38
	5	5/17 18	Controls			445	59.2	24.2	
III	10	6/21	Stannous tartrate Pelletierine HCl	0.8 0.2	7/25	1,166.0	97.4	2.0	89.24
	10	6/21	Controls			1,078.8	157.8	18.6	
IV	9	10/10	Stannous tartrate Pelletierine HCl	1.0 0.2	11/21	1,384.8	81.8	.22	99.82
	9	10/10	Controls			1,350.8	112	12.3	
V	9	11/9	Tin oleate	2.5	11/29	807.1	109.9	7.9	32.38
	9	10/9	Stannous tartrate Pelletierine HCl	1.0 0.2	11/29	829.7	87.1	2.1	80.67
	9	10/9	Controls			802.9	132.5	11.7	
VI	10	11/3	Stannous tartrate Pelletierine HCl	0.5 0.15	12/6	477.7	60.6	1.0	95.43
	10	11/3	Controls			483.3	108.5	21.9	
VII		(1940)			(1940)				
	10	1/29 30	Stannous tartrate Pelletierine HCl	0.8 0.2	2/27	770.4	76.4	2.4	92.0
	10	1/29 30	Tin oleate Pelletierine HCl	2.0 0.2	2/27	747.2	92	1.0	96.66
	10	1/29 30	Stannous oxalate Pelletierine HCl	0.5 0.2	2/27	778.3	76.9	0.9	97.0
	10	1/29 30	Stannous oxide hydrate Pelletierine HCl	0.5 0.2	2/27	765.5	77.2	1.4	95.33
	10	1/29 30	Metallic tin (powdered) Pelletierine HCl	1.0 0.2	2/27	748.5	95.7	5.7	81.0
	10	1/29 30	Controls			778.8	72	30.0	
VIII	10	3/14 15	Tin oleate Pelletierine HCl	2.0 0.2	4/9	791.8	73.9	2.6	88.18
	10	3/14 15	Tin oleate Pelletierine HCl	1.0 0.1	4/9	768.4	100.2	3.5	84.09
	10	3/14 15	Stannous oxalate Pelletierine HCl	0.5 0.2	4/9	801.6	42.8	0.8	96.36
	10	3/14 15	Stannous oxalate Pelletierine HCl	0.25 0.1	4/9	791.8	110.5	5.7	74.09
	10	3/14 15	Stannous oxide hydrate Pelletierine HCl	0.4 0.1	4/9	794.3	64.2	5.0	77.27
	10	3/14 15	Controls			789.4	71	22.0	

*These birds were infected with 50 cysticercoids each.
†All birds were necropsied 14 days after treatment.

TABLE V—Efficacy of Stannous Tartrate Plus Synthetic Pelletierine Hydrochloride for the Removal of Hymenolepis Carioca from Experimentally Infected Chickens*

Date of Infection (1939)	No. of Birds in Experiment	Date of Treatment (1940)	Drugs Employed	Dose (Gm.)	Mean Weight at Time of Treatment (Gm.)	Mean Gain in Weight During 4 Days Following Treatment (Gm.)	Date of Necropsy (1940)	Tapeworms Recovered at Necropsy (Mean No.)	Efficacy of Treatment (Per Cent)
11/9	4	1/30	Stannous tartrate Pelletierine HCl	1.0 0.2	907.5	20	2/13	0.75	99.85
11/9	5		Controls				1/30	532.6	
12/5	5	2/8	Stannous tartrate Pelletierine HCl	0.5 0.1	1,063.5	94	2/23	7.6	99.17
12/5	4		Controls				2/8	917.0	

*These birds were infected with approximately 1,000 cysticercoids each.

TABLE VI—Effects on Young Chickens of Large Doses of Tin Compounds and of Tin Compounds Plus Synthetic Pelletierine Hydrochloride

Band No.	Weight (Gm.)	Treatment		Results
		Substance	Dose (Gm.)	
1589	1,010	Stannous tartrate	2.0	Reduced appetite for one day
		Pelletierine HCl	0.4	
1647	1,355	Stannous tartrate	2.0	Partial inappetence and incoordination of leg movements lasting one day
		Pelletierine HCl	0.4	
1559	1,172	Stannous tartrate	3.0	Partial inappetence and incoordination of leg movements lasting one day
		Pelletierine HCl	0.6	
1618	1,260	Stannous tartrate	3.0	Partial inappetence and reluctance to move for one day
		Pelletierine HCl	0.6	
1551	1,260	Stannous tartrate	3.5	Appetite reduced for two days; legs paralyzed on third day; recovery complete by fourth day
		Pelletierine HCl	0.7	
1683	1,230	Stannous tartrate	3.5	Appetite reduced for two days; some leg weakness and incoordination on third day; recovery complete by fourth day
		Pelletierine HCl	0.7	
1108	930	Stannous oxalate	1.75	Partial inappetence and incoordination of leg movements lasting two days
		Pelletierine HCl	0.5	
1159	1,240	Stannous oxalate	1.75	Slight incoordination lasting one day
		Pelletierine HCl	0.5	
1144	1,100	Stannous oxalate	3.5	Partial inappetence and incoordination on first day; complete paralysis on second and third days; died on fourth day; catarrhal enteritis at necropsy
		Pelletierine HCl	1.0	
1102	1,120	Stannous oxalate	3.5	Slight incoordination on first day; dead on second day; catarrhal hemorrhagic enteritis at necropsy
		Pelletierine HCl	1.0	
1191	1,060	Stannous oxalate	3.5	No symptoms of intoxication
1074	970	Stannous oxalate	3.5	Slight inappetence lasting until death on fourth day; catarrhal enteritis at necropsy
1113	1,142	Tin oleate	5.0	No symptoms of intoxication
		Pelletierine HCl	0.5	
1041	1,380	Tin oleate	5.0	No symptoms of intoxication
		Pelletierine HCl	0.5	
1153	1,010	Tin oleate	10.0	Slight leg weakness on first day; paralyzed on second; died on third; severe cartarrhal enteritis at necropsy
		Pelletierine HCl	1.0	
1147	980	Tin oleate	10.0	Inappetence on first and second days; paralysis on third day; died on fourth day; hemorrhagic-catarrhal enteritis at necropsy
		Pelletierine HCl	1.0	
1197	925	Tin oleate	10.0	No symptoms of intoxication noted
1132	1,100	Tin oleate	10.0	No symptoms of intoxication noted

removal of *R. cesticillus* from experimentally infected chickens. By adding small amounts of synthetic pelletierine hydrochloride to any one of various tin compounds, a mixture was obtained which effectively removed *R. cesticillus* and *H. carioca* from experimentally infected chickens, as indicated by the data included in tables I, II, IV and V. Synthetic pelletierine hydrochloride administered in doses of .05 to 0.3 Gm. proved effective for the removal of *R. tetragona*,[1] but when used alone this substance proved quite ineffective for the removal of *R. cesticillus* from chickens. Obviously, a synergistic action was obtained by administering the tin compounds and the synthetic pelletierine together, since each was weakly taeniacidal when used alone. This result is contrary to a common view that a mixture of two anthelmintics is less effective than the pure substances used separately. At present no explanation, supported by adequate data, can be advanced that would account for the increased taeniacidal value of the mixtures under consideration.

It may be noted that the habitat of each of the three species of tapeworms discussed in this paper is in a different part of the intestinal tract, and that the species also differ in other respects, such as size and relative depth at which the scolex is attached in the intestinal wall. *H. carioca* occurs in the duodenum where the villi are very long; the strobila is delicate and usually a good part of it is interlaced among the villi, and the scolex is very deeply buried, occurring at or near the bottom of the crypts of Lieberkuhn. *R. cesticillus* occurs in the jejunum, the coarse strobila lying free in the intestinal lumen and the scolex being less deeply embedded among the villa than is the case with *H. carioca*. *R. tetragona*,* which occurs in the ileum, also has a coarse strobila, but the scolex of this tapeworm is attached very superficially. Since tin-pelletierine mixtures

*The question arose as to whether synthetic pelletierine hydrochloride mixed with a tin compound retained its efficacy for the removal of *R. tetragona*. A naturally infected bird weighing 1,435 Gm. was treated with a mixture of 1 Gm. of stannous tartrate and 0.2 Gm. of synthetic pelletierine hydrochloride. A total of 136 complete specimens of *R. tetragona* were passed subsequent to treatment. At necropsy two weeks after treatment no tapeworms of this species were present.

have proved effective for the removal of these tapeworms, it is possible that these mixtures will prove equally effective against the other species of tapeworms encountered in chickens. Naturally, this point will have to be verified by controlled tests.

The limited information which is available at the present time suggests the possibility that tin preparations may be effective against other species of tapeworms occurring in domestic animals. This seems reasonable, since metallic tin was used in the past with some degree of success against the tapeworms of man* and Lepinay[9] has indicated that treatment with pure oxide of tin removed several taenioid scolices from dogs.

While these investigations were in progress, the authors were consulted by Mr. J. B. Fox of Washington, D. C., in regard to an effective taeniacide for use in a duck hawk *(Falco peregrinus anatum)* which was in poor condition. It was suggested that he try a mixture of stannous tartrate and synthetic pelletierine hydrochloride, which actually proved to be effective although somewhat toxic.† Mr. Fox reported that following treatment the bird gained weight and improved in general condition.

These limited data suggest that tin soaps mixed with synthetic pelletierine may prove useful for the removal of tapeworms from a variety of hosts.

SUMMARY AND CONCLUSIONS

The tests herein reported show that inorganic and organic tin compounds are of slight value for the removal of tapeworms from experimentally infected chickens. Mixtures containing a small amount of synthetic pelletierine hydrochloride and one of the following tin compounds, namely, tin oleate, stannous oxalate, stannous oxide hydrate or stannous tartrate effectively removed *Raillietina cesticillus* from chickens infected with 50 cysticercoids each. Mixtures of 0.3 to 1 Gm. of stannous tartrate and .07 to 0.2 Gm. of synthetic pelletierine hydrochloride were administered to 58 birds with an average indicated efficacy of 86.8 per cent. A mixture of synthetic pelletierine hydrochloride and stannous tartrate was better than 95 per cent effective for the removal of *Hymenolepis carioca* from nine chickens which had been experimentally infected with 1,000 cysticercoids each. Available information suggests that the therapeutic index is high enough to offer a sufficiently wide margin of safety for these mixtures. Accordingly, further work seems justified to determine the most effective and economical of these mixtures for use in chickens.

References

[1]Harwood, P. D., and Guthrie, J. E.: Tests with miscellaneous substances for the removal of tapeworms from chickens. J.A.V.M.A., xcvii (1940), 762, pp. 248-253.

[2]Paracelsus, A. P. T.: Die Bücher u. Schrift. herausg. Huserum (1589).

[3]Alston, C.: Powder of tin an anthelmintic medicine. Med. Essays & Obser. Soc. Edinburgh, v (1742), pp. 89-92.

[4]Richter, G. A.: Ausfürliche Arzneimittellehre Handbuch für praktische Aerzte (iv, 1829, pp. 548-558).

[5]Dommes, reported by Dyson, G. W.: The compounds and uses of tin. Phar. J. & Phar., cxxvi (1931), pp. 299-300 and 498-499.

[6]Dochevskii, I. I.: (Tin as a remedy for tapeworms) (Russian text). Vrch. Gaz., ix (Nov. 2, 1902), Therap. Otdel, p. 85.

[7]Delektorskii, N. I.: (Tin as a remedy against tapeworms in children) (Russian text). Terapiia (S. Peterh.) i (5), pp. 168-171.

[8]Fedorov, P. I.: (Anthelmintic action of tin) (Russian text). Sibirsk. Vrach Viedom., iii (1905), 4, pp. 55-57.

[9]Lepinay, L.: Un noveau toeniafuge. Rev. Path. Comp., xxxiii (Avr.), pp. 545-547.

[10]Anonymous: Tin, Tapeworms, and Tush. J.A.M.A., cxiii (11), p. 1034.

[11]Frouin, A., and Gregoire, R.: Action de l'étain metallique et de l'oxyde de l'étain dans les infections à staphylocoque. Compt. Rend. de l'Acad. des Sci., clxiv (May 1917), pp. 794-797.

[12]Compton, A.: The treatment of staphylococcal infections by stannoxyl: furunculosis and acne. Lancet, i (1918), 3, pp. 99-100.

*Recently an editorial, "Tin, Tapeworms, and Tush," appeared in *The Journal of the American Medical Association*[10] in which the author ridiculed the idea that tin preparations might prove of value as taeniacides in man. Apparently the author of this editorial was not familiar with much of the information contained in the literature, which indicates that tin may be approximately as effective as some of the widely used remedies, and possibly much less dangerous.

†A few hours after a mixture of 0.5 Gm. of stannous tartrate and 0.1 Gm. of synthetic pelletierine hydrochloride was given to the hawk, eight complete tapeworms of the species *Cladotaenia foxi* McIntosh, 1940 (Proc. Helminth. Soc. Wash., vii (2), pp. 71-74) were eliminated. According to Mr. Fox, the treatment was relatively severe and the bird was off feed for one day. Possibly a smaller dose would have been as effective and less toxic. Without our advice a dose of .75 Gm. of stannous tartrate and .15 Gm. of synthetic pelletierine was given to a second hawk, which died in a few hours. At necropsy one specimen of *C. foxi* was present.

[13]Wright, C. S.: The treatment of skin infections with combined tin proteinate and Brewer's yeast. Med. Rec., cxlvii (10), pp. 453-455.

[14]Sellier, M. J.: L'emploi de l'étain metallique et des sels d'étain en médecine vétérinaire. Rec. de Méd. Vét., v (1927), pp. 146-150.

[15]Kolmer, J. A., and Harkins, M. J.: Stannoxyl in the chemotherapy of experimental staphylococcus infections. J. Chemotherapy, viii (1931), pp. 6-9.

[16]Kolmer, J. A., Brown, H., and Harkins, M. J.: A note on tin compounds in the chemotherapy of experimental staphylococcus infections. J. Phar. & Exp. Ther., xlii (1931) pp. 515-519.

[17]Klein, J. E.: Stannoxyl in the treatment of chronic recurring osteomyelitis. Annals Surg. xcvi (1932), pp. 1032-1035.

[18]Klein, J. E.: Metallotherapy in the conservative treatment of chonic osteomyelitis. Arch. Pediatrics, liii (1936), pp. 476-489.

[19]Levy, S.: Ueber die behandlung von furunkeln und hordeolum mit zinn. Deut. med. Wochnschr., lii, pp. 1303-1304.

[20]Schlesinger, J.: Zinnbehandlung bei hordeolosis. Zeit. f. Augenh., lxxiii, pp. 175-179.

[21]White, T. P.: Ueber die wirkungen des zinns auf den thierischen organismus. Arch. f. Exp. Path. u. Pharmakol., xiii, pp. 53-69.

[22]Ungar, E., and Bödlander, G.: Ueber die toxischen wirkungen des zinn, mit besonderer berücksichtigung der durch den gebrauch verzinnter conserven buchsen der gesundheit drohenden gefahren. Z. Hyg., ii, pp. 241-296.

[23]Salant, W., and Smith, C. S.: The toxicity of tin. J. Phar. & Exp. Ther., v (1914), p. 517.

[24]Salant, W., and Rieger, J. B.: Further observations on the toxicity of tin. Proc. Soc. Exp. Biol. & Med. (New York), xi (1914), pp. 178-179.

[25]Salant, W., Rieger, J. B., and Treuhardt, E. L. P.: Absorption and fate of tin in the body. J. Biol. Chem., xvii (1914), pp. 265-273.

[26]Salant, W., Rieger, J. B., and Treuhardt, E. L. P.: The distribution and elimination of zinc and tin in the body. J. Biol. Chem., xxxiv, 2 (1918).

[27]Salant, W.: The pharmacology of heavy metals. J. Indust. Hyg., ii (1920), pp. 72-78.

[28]Schryver, S. B.: Some investigations on the toxicology of tin with special reference to the metallic contamination of canned foods. J. Hyg., ix (1909), pp. 253-263.

[29]Goss, B. C.: Inhibition of digestion of proteins by adsorbed tin. J. Biol. Chem., xxx (1917), pp. 53-60.

[30]Hanzlik, P. J., McIntyre, M., and Presho, E.: Experimental plumbism: therapeutic efficiency of some agents and comparative toxicity of other metals. Proc. Soc. Exp. Biol. & Med., xix (1922), pp. 192-193.

[31]Bertrand, G., and Ciurea, V.: L'étain dans l'organisme des animaux. Compt. rend. Acad. Sci. Paris, cxcii (1931), pp. 780-782.

[32]Harwood, P. D.: Reproductive cycles of *Raillietina cesticillus* of the fowl. Livro Jubilar Prof. Travassos, Rio de Janeiro, Brazil, III (1938), pp. 213-219.

[33]Ackert, J. E.: The longevity of fowl tapeworms. J. Parasitol., vii (1921), pp. 198-199.

[34]Chandler, A. C.: Observations on the life cycle of *Davainea proglottina* in the United States. Tr. Amer. Micr. Soc., xlii (1923), pp. 144-147.

Tentative Pathologic Nomenclature

for the Disease and/or the Disease Complex Variously Designated as Fowl Leucemia, Fowl Leucosis, Range Paralysis, Fowl Paralysis, Iritis, Lymphomatosis, Lymphocytoma, Neurolymphomatosis, Leucotic Tumors, Leucemoid Diseases, etc.*

AVIAN LEUCOSIS COMPLEX

1) Lymphomatosis { neural[1] / visceral[2] / ocular[1] / osteopetrotic[3] }

2) Leucosis { erythroblastic[4] / granuloblastic[4] }

It is understood that the various forms may be characterized by aleucemic, subleucemic, or leucemic blood pictures and should be further classified on this basis. Each pathologic form occurring in a specimen should be noted.

OTHER TUMORS

Myelocytoma
Sarcoma
Etc.

*Adopted by a conference of investigators in this field (July 15-17, 1940, at East Lansing, Mich.) with the object of facilitating uniformity in terminology and interpretation of data. This scheme does not imply classification on a basis of etiology. Submitted by Erwin Jungherr, D.M.V., chairman of the Pathology Committee, Experiment Station Collaborators of the U. S. Regional Poultry Research Laboratory, East Lansing, Mich.

References

[1]Pappenheimer, A. M., Dunn, C. C., and Cone, V.: A study of fowl paralysis (*Neurolymphomatosis gallinarum*). Storrs Agr. Exp. Sta. Bul. 143 (1926).

[2]Johnson, E. P.: A study of lymphomatosis of fowls. Virginia Agr. Exp. Sta. Bul. 44 (1932).

[3]Jungherr, E., and Landauer, W.: A condition resembling osteopetrosis (marble bone) in the common fowl. Storrs Agr. Exp. Sta. Bul. 222 (1938).

[4]Furth, J.: Observations with a new transmissible strain of leucosis (leucemia) of fowls. J. Exp. Med., liii (1931), pp. 243-267.

The Use of Trypaflavin in the Infusion Therapy for Streptococcic Mastitis*

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IN A PREVIOUS paper,[1] the writer presented his results with the acridine-containing compound, entozon,† in the treatment of streptococcic mastitis by udder infusion. The interest shown by the profession in this method of treatment of mastitis has prompted the writer to prepare this preliminary report of results with trypaflavin, a brand of acriflavine.

REVIEW OF STECK'S EXPERIMENTS

Literature pertaining to the use of the acridines, rivanol, uberasan and entozon, for mastitis was reviewed in the previous paper[1]; however, the investigations of Steck of Switzerland were mentioned only briefly and, therefore, a review of his work will be presented in order that practitioners in America may become familiar with his promising method for the treatment of streptococcic mastitis.

In 1929, when criticism of the acridine-infusion therapy for mastitis had reached its peak, Steck[2, 3, 4, 6] began a thorough study of this problem. He compared rivanol, uberasan, entozon and trypaflavin with regard to their lethal effects upon *Streptococcus agalactiae* and their irritating properties on the udder tissues. He found that in concentrations having equal irritative action, trypaflavin was approximately five times more lethal for the streptococci in fresh secretions obtained from infected cows than either rivanol or entozon, and approximately three times more so than uberasan. These observations led him to use trypaflavin in his further studies of the infusion therapy.

During the course of his investigations, Steck[3, 5] came to the conclusion that the principal foci of infection with the mastitis streptococci are in the gland cistern and large ducts, rather than in remote areas of the parenchyma and that, therefore, a more efficient sterilization should result from treating the cistern region more intensively than the parenchyma. To accomplish this he developed his so-called method I, in which the cistern and large ducts are exposed to a solution of trypaflavin, approximately nine times stronger than that infused into the parenchyma. The technic is as follows: 12 cc. of a 1 per cent trypaflavin solution is added to 1,750 cc. of sterile water, giving approximately a 1:14,000 dilution; 250 cc. of this dilute solution is infused into the milked-out quarter as a preliminary rinse. After a light massage, this is milked out and replaced by 1,100 cc. of the dilute solution, which leaves 400 cc. in the container. Without removing the needle, 22 cc. of the 1 per cent stock solution is added to the remaining 400 cc., giving a final concentration of trypaflavin of 1:1,600, for infusion into the cistern. Two minutes after the injections are completed, 500 cc. if possible, but not more, should be milked out.

Of 175 quarters treated by method I,[6] 131, or 75 per cent, were cured by a single treatment. The average milk reduction, estimated ten days after infusion, was 20 per cent. While in some instances the permanent reduction of secretion was negligible, in others it was much higher than the average. Steck attributed this to the fact that frequently, due to the marked ability of the udder tissues to retain infused fluids, it was not possible to regain the 400 cc. of concentrated trypaflavin solution infused last.

Following a series of experiments designed to increase the volume of regainable fluid, method II[7] was evolved. This method differed

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*From the Division of Veterinary Science, University of California; assistance in the preparation of this report was furnished by the personnel of WPA Project O.P. No. 665-08-3-29.

†Entozon is manufactured by the I. G. Farben Industry of Germany and is not available at the present time in this country.

[1]Schalm, O. W.: The treatment of streptococcic mastitis by infusion of the udder with entozon. J.A.V.M.A., xcvii (July 1940), pp. 20-27.

[2]Steck, W.: The control of *Streptococcus agalactiae* mastitis. Proc. 12th Int. Vet. Cong., ii (1934), pp. 494-505.

[3]*Ibid.:* A new method for the eradication of *Streptococcus agalactiae* mastitis in a herd. Cornell Vet., xxv (1935), pp. 1-5.

[4]*Ibid.:* An experimental comparison of different acridine derivatives for the treatment of mastitis. (Title trans.) Schweiz. Arch. Tierheilk., lxxvi (1934), pp. 559-569.

[5]*Ibid:* A method for the eradication of mastitis in infected herds. (Title trans.) Schweiz. Arch. Tierheilk., lxxvi (1934), pp. 504-509.

[6]*Ibid.:* Radical control of mastitis during lactation by means of acridine-therapy. (Title trans.) Schweiz. Arch. Tierheilk., lxxix (1937), pp. 1-20.

[7]*Ibid.:* A guide to the treatment of mastitis. (Title trans.) Schweiz. Arch. Tierheilk., lxxviii (1936), pp. 42-46.

from the first only in that sucrose was added to the concentrated solution infused last. The writer has failed to find in Steck's many papers on the infusion therapy a statement of the actual concentration of sucrose employed. However, in one publication[6] a table is given which shows the effect of 20 and 40 per cent dextrose and 20 per cent sucrose upon the regainable fluid. From this it may be presumed that 20 per cent sucrose is the concentration used. Method II was reported to be as efficient as method I in sterilizing the udder, while the reduction of secretion was somewhat less, averaging 18 per cent.

Eventually Steck dispensed with infusion of the parenchyma and gave only an amount of highly concentrated trypaflavin-sucrose solution sufficient to fill the cistern region. This he called his method III.[8, 9, 10]

By the end of 1938, method III had been used on 5,000 infected quarters, producing 75 per cent cures by one infusion and 90 per cent by two treatments, with the milk loss negligible. Briefly, method III is as follows: 200 cc. of a dilute solution of trypaflavin (presumably 1:14,000) is infused into the milked-out quarter, followed by infusion of 200 cc. of concentrated trypaflavin (over .07 per cent) in a hypertonic sucrose solution (presumably 20 per cent). After a short, light massage, milk out the infused fluid into a graduated container. A second infusion of concentrated trypaflavin-sucrose solution is made, a volume being used equal to the amount regained from the first infusion plus 50 cc., but never more than 400 cc. should be given. Steck states that this method may be employed at any time during lactation and it may be used on the dry udder providing the gland has not started to prepare for its next lactation. Ten to 14 days after treatment, samples should be taken and if streptococci are still present, a second treatment should be given; if this fails to cure, a third treatment should be administered, which should include two separate injections of the 400 cc. of concentrated trypaflavin-sucrose solution.

EXPERIMENTAL PROCEDURE

The acridine derivative used in the experiments reported below was trypaflavin,* a brand of acriflavine. Trypaflavin corresponds to neutral acriflavine or acriflavine base of the United States Pharmacopoeia.[11] Chemically it is a mixture of 2, 8 diamino- 10-methylacridinium chloride and 2, 8 diamino-acridine. It is a brownish-red, crystalline powder which is soluble in water. Solutions can be sterilized by autoclaving without decomposition, but they are sensitive to light and, therefore, should be kept in amber bottles.

Infusion Apparatus and Infusion Technic.—The method employed at the California Station in giving the infusion and the apparatus used were the same as previously described by the writer.[1] The infusion fluid was always made up at the dairy immediately prior to use. Boiled, filtered tap water was used as a solvent for the trypaflavin and, whenever possible, was heated to between 30 and 35° C. before use; however, at times it was not convenient to do this, and it had to be infused at a temperature of approximately 22° C. Since no ill effects were observed that could be attributed to the temperature of the fluid, it may be assumed that a warm infusion fluid is not essential.

Mastitis Diagnosis.—Four tests for the detection of mastitis were employed throughout this study. The strip-cup test and brom-cresol-purple (BCP) paper test for pH were used on the fore milk, after which the teats were washed with a chlorine solution and approximately 20 cc. of milk was collected in sterile test tubes for bacteriologic study. The milked-out quarters were palpated for fibrosis and the individual quarters were classified in a manner similar to that described by Udall.[12] Palpation numbers of 1, 2, 3 and 4 were used. Quarters classified as number 1 were regarded as normal; those classified as number 2 exhibited a distinct but not extensive firmness of the cistern area and were regarded as suspicious; those classified as number 3 showed unquestionable and fairly extensive fibrosis,

*Obtained from the Winthrop Chemical Company.

[8] *Ibid.:* Treatment of the milk cistern with zysternal. A new method to cure mastitis infection during lactation. (Title trans.) Schweiz. Arch. Tierheilk., lxxviii (1936), pp. 470-472.

[9] *Ibid.:* The treatment of mastitis with trypaflavin. (Title trans.) Arch. Wiss. u. Prakt. Tierheilk., lxxiii (1938), pp. 360-361.

[10] *Ibid.:* A system to eradicate *Streptococcus agalactiae* mastitis from the milk cow based on bacteriological diagnosis and intensive treatment of the cistern zysternal. (Title trans.) Verlag Paul Haupt, Bern-Leipzig.

[11] Personal communication from Dr. Charles H. Fanslau of the Winthrop Chemical Company.

[12] Udall, D. H., Johnson, S. D., and Ferguson, J. : The control of mastitis in New York State. Vet. Rec., 1 (1938), pp. 1417-1932.

usually limited to the lower half of the udder; while quarters classified as number 4 showed a marked firmness throughout. In some instances this firmness was produced by swelling of the quarter as a result of an acute inflammatory reaction, but in most cases it represented an extensive fibrosis of long standing.

For the bacteriologic investigations, 1 cc. of a 1:100 dilution of milk was plated with veal infusion agar containing from 5 to 7 per cent horse or ox blood and incubated at 37° C. for 48 hours. The remainder of the whole milk was incubated overnight, after which smears were prepared by the Broadhurst-Paley[13] method for microscopic examination. Colonies suspected of being streptococci were fished from plates corresponding to smears of incubated milk in which streptococci were found. Gram-stained smears were prepared from these cultures after overnight incubation and were examined microscopically for morphology and purity. Cultures proving to be streptococci were transferred to essential differential media for classification. The isolation of *S. agalactiae*, *S. uberis* or *S. dysgalactiae* was considered as a diagnosis of infection and the quarter was treated.

All treated cows were sampled weekly for bacteriologic study for at least one month after treatment, and then monthly thereafter as long as they were kept in the herd, with the exception that during the dry period testing was done less frequently. A cow was considered cured if the species of streptococcus isolated prior to treatment was not found again during the current lactation period or, in the cases of cows treated while being dried up, during their subsequent lactation period. The isolation of streptococci of a different species from that found prior to treatment or the isolation of the same species again in subsequent lactation periods was regarded as a reinfection of a cured case. However, to reduce the occurrence of reinfection, treated cows were kept segregated from known-infected animals in the herd.

[13]Broadhurst, J., and Paley, C.: A single-dip stain for the direct examination of milk. J.A.V.M.A., xcv (May 1939), pp. 525-526.

Results with Aqueous Solutions of Trypaflavin

In the preliminary trials with trypaflavin, a 1:4,000 aqueous solution was used on twelve cows having a total of 29 infected quarters, 28 of which were shedding *S. agalactiae* and one, *S. dysgalactiae*. In three of the cows only the quarters proved to be shedding streptococci were infused, while with the remaining nine animals, all quarters were treated.

The method of treatment was as follows: 200 cc. of a 1:4,000 trypaflavin solution was infused into the milked-out quarters as a preliminary rinse; after a short, light massage, this was milked out and replaced by the principal infusion, the volume of which varied between 800 and 1,500 cc., depending upon the capacity of the quarter. Eight cows were lactating when treated and with them the principal infusion was left in the udder for periods of time varying between 15 minutes and three hours. In milking out the infusion fluid, usually not more than 100 to 150 cc. could be regained, the remainder being retained by the tissues. Four cows were being dried up when treated. With one of these the principal infusion was left in for one hour in the first treatment and for three hours in a repeat treatment. With two cows the principal infusion was left in overnight and with one animal it was not milked out again. One week after the treatment was given, the cows were sampled and if streptococci were still present, a second infusion was made two weeks after the first; if a third was necessary, it was administered four weeks after the first. The histories of the cows, method of treatment and results obtained are presented in table I.

Since the infusions were continued until streptococci were no longer found in the udder secretion, it may be assumed that a 100 per cent eradication of infection was obtained. However, this success was overshadowed by the excessive irritation which accompanied the infusions and which led

TABLE I—Results Obtained with a 1:4,000 Aqueous Solution of Trypaflavin in the Treatment of Streptococcic Mastitis

Cow No.	Mos. of Lact.	Approx. Duration of Infection (Mos.)	Method of Treatment					Results			Conclusions
			Number of Infect. Quarters	Number Quart. Treated	Times Treated	Volume of Principal Infusion (cc.)	Hours Left In	Number Weekly Tests After Treatment Negative for Strep.	Number Quarters Obliterated by Treatment	Number Quarters Partially Damaged	
23	1	3	3	4	1	800	1	26	0	0	Satisfactory cure
24	1	9	2	2	1	1,200	½	4†	1	0	Cured, but one quarter damaged
25	1	3	2	4	3	800 to 1,500	2	22†	1	1	Cured, but two quarters damaged
26	1	-	4	4	3	800 to 1,500	2, 2 and 3	16†	0	2	Cured, but two quarters damaged
27	1	10	2	4	1	1,200	1¼	14†	1	0	Cured, but one quarter damaged
28	1	11	1	1	1	1,000	¼	21	0	0	Satisfactory cure
29	1	9	2	2	1	1,000	½	16	0	2	Cured, but two quarters damaged
30	2	3	1	4	1	1,000	1	24	0	0	Satisfactory cure
31	Being dried	7	4	4	2	800	1, 2 and 3	5‡	2	2	Severe damage to all quarters
32	Being dried	24	3	4	3	600 to 1,500	0 N*	4‡	2	1	Severe damage to 3 quarters
33	Being dried	12	2	4	1	800	0 N*	8‡	2	2	Severe damage to all quarters
34	Being dried	3	4	4	1	1,000 to 1,500	One week	30	0	0	Satisfactory cure

*O N=Overnight. †Cow sold because of damaged udder. ‡Cow sold shortly after freshening.

to permanent damage of one or more quarters of eight of the twelve cows. The severest injury occurred among the cows treated at the end of their lactation periods. Three of them, Nos. 31, 32 and 33, treated two, three and one times, respectively, during their sixth month of gestation, exhibited marked udder damage. At calving, each cow had a well-developed, symmetrical udder, but in each case no milk could be obtained from two quarters and only a small quantity could be gotten from the remaining quarters. These cows were slaughtered and their udders were obtained for study. In each udder it was found that the parenchyma of all quarters was well developed but that the milk could not be removed because of a partial or complete obliteration of the lumens of the teats and large ducts by scar tissue. In contrast to these results are the observations on cow 34, which was also treated at the end of her lactation period and in which the infusion fluid was not milked out again. This cow had exhibited bloody udder contents when sampled one week after treatment. When she calved three months later, there were no signs of udder damage and she has milked normally throughout the subsequent lactation period. Five of the eight cows treated during lactation developed visible udder damage following the infusion. In each of three cows, the teat lumen of one quarter became occluded by scar tissue, while five other quarters on three cows underwent partial atrophy. Out of a total of 41 quarters infused with a 1:4,000 aqueous solution of trypaflavin, 19, or 46.3 per cent, were visibly damaged. The trypaflavin was apparently used in too high a concentration for the period that it was allowed to remain in the udder. Udall[14] has used a 1:4,000 concentration for shorter intervals

[14]Udall, D. H.: The Practice of Veterinary Medicine (3rd ed., 1937).

and Stableforth and Scorgie[15] have reported favorable results with a 1:10,000 concentration left in the udder for five minutes.

In order to determine the proper concentration of trypaflavin and most favorable time interval for it to be left in the udder to obtain the most efficient sterilization and at the same time produce the least tissue irritation, the following experiment was made: Three cows, Nos. 35, 36 and 37, which had been infected for 12, 24 and 6 months, respectively, were selected for treatment. Cows 35 and 36 were shedding *S. agalactiae* from all quarters, while cow 37 was infected with *S. uberis* in its LR* quarter and with *S. agalactiae* in the other three. The two right quarters of each cow were infused with a 1:10,000 concentration of aqueous trypaflavin, while the left quarters received a 1:4,000 concentration. The volume of principal infusion given was just sufficient to fill out the quarter without stretching it too tensely, and it was left in cow 35 for five minutes; in cow 36, for ten minutes; and in cow 37, for 15 minutes. The histories of the cows, the method of treatment and the results obtained are shown in table II.

In these comparative tests, it was observed that the 1:10,000 concentration was as effective as the stronger one in removing the streptococci and that it was much less irritating. With both concentrations four quarters were given one infusion, while one quarter required three and another four treatments to eradicate the infection.

Within a few hours after infusion of the 1:4,000 concentration, the quarters were swollen and firm, while those receiving the 1:10,000 concentration showed little or no change. In both instances the milk was stained yellow for several days, visible particles appeared in the foremilk and it was alkaline to the BCP test. With quarters receiving the weaker solution, the visible particles were limited to the foremilk and disappeared again after seven to ten days, while with the quarters infused with the stronger solution, the particles were usually larger, frequently were found throughout the milk and persisted for ten days or longer. In three quarters, the LF and LR of cow 35 and the LF of cow 36, the 1:4,000 concentration failed to destroy all of the streptococci on the first infusion, and this was followed by an acute flare-up of the infection. It appeared that the irritation produced by the infusion fluid so lowered the resistance of the tissues that the surviving streptococci were able to develop unhampered. The inflammation resulting in the LF of cow 35 was so severe that it was impossible to infuse fluid for another treatment; when the swelling subsided, the teat had become occluded by scar tissue. The LR of cow 35 also developed a persistent swelling which was associated with shedding of large numbers of *S. agalactiae*. Three treatments were given before a cure was produced.

In the second and third infusions a 1:10,000 concentration was used and it was left in for ten and 15 minutes, respectively. The LF of cow 36 developed a persistent swelling after the first infusion with the 1:4,000 concentration, and this was accompanied by an increase in the number of both streptococci and micrococci in the milk. A total of four infusions of the 1:4,000 concentration was necessary to eliminate the streptococci from this quarter. This caused the development of a partial stricture of the teat lumen which became complete during the subsequent dry period.

The effect of the treatment on milk production was studied. It was found that infusions with the 1:10,000 concentration were followed by a decrease in production varying from 0 to 18 per cent when one treatment was given, and between 12.5 and 32 per cent when three or four infusions were necessary. With the quarters receiving the 1:4,000 concentration, a decrease varying from 55 per cent followed one infusion, and when three or four treatments

*RF=right front; RR=right rear; LF=left front; LR=left rear.

[15] Stableforth, A. W., and Scorgie, N. J.: Entozon and acriflavine for the treatment of chronic contagious bovine mastitis. Vet. Rec., xlvi (1934), pp. 663-676.

TABLE II—A Comparative Study with 1:4,000 and 1:10,000 Aqueous Solutions of Trypaflavin Left in the Udder for Short Intervals of Time

Cow No.	Mo. of Lact.	Approx. Duration of Infect. (Mos.)	Qtr.	Treatment Data					Results of Various Tests Before and After Treatments*											
				Number Infusions Required	Dilution of Trypaflavin Used	Volume† Infused (cc.)	Min. Left in Qtr.	Fluid Regained (%)	Strip Cup		BCP		Palpation Score		Col. Strep. per cc. of Foremilk		Volume of Milk (cc.)		Decrease in Product. (%)	Conclusions
									B	A	B	A	B	A	B	A	B	A		
35	7	12	RF	1	1:10,000	800	5	31.1	0	0	0	0	1	1	40,000	0	830	680	18.0	Satisfactory cure
			RR	1	1:10,000	1,000	5	45.0	0	0	0	0	1	1	10,000	0	2,050	2,180	0.0	Highly satisfactory cure
			LF	1	1: 4,000	800	5	30.0	0	+	0	+	1	4‡	45,000	Very high	870	0	100.0	Severe injury
			LR	3	1: 4,000 1:10,000 1:10,000	1,000 1,000 1,200	5 10 15	36.5 30.0 26.5	0	0	0	0	2	2	18,600 150,000 21,500	0	2,970	1,030	65.4	Cured but production greatly decreased
36	10	24	RF	4	1:10,000	1,000 1,200 1,000 1,000	10 10 10 10	23.0 35.4 27.5 30.0	0	0	0	0	3	3	39,000 19,500 5,000 8,000	0	1,000	680	32.0	Satisfactory cure
			RR	3	1:10,000	1,000 1,200 1,000	10 10 10	32.0 41.5 35.0	0	0	0	0	3	3	26,000 26,000 32,500	0	1,600	1,400	12.5	Satisfactory cure
			LF	4	1: 4,000	1,000 1,200 1,200 1,200	10 10 10 15	34.0 42.5 33.1 24.5	0 10	0	0	0	3	4‡	21,000 Very high do. 5,500	0	800	120	85.0	Severe injury
			LR	1	1: 4,000	1,000	10	42.5	0	0	0	0	3	3	65,000	0	700	800	0.0	Highly satisfactory cure
37	9	6	RF	1	1:10,000	800	15	10.0	Pus	0	+	0	4	2	25,000	0	200	230	0.0	Highly satisfactory cure
			RR	1	1:10,000	800	15	26.3	Pus	0	+	0	4	2	52,000	0	400	420	0.0	Highly satisfactory cure
			LF	1	1: 4,000	775	15	13.8	Pus	0	+	0	4	2	80,000	0	300	135	55.0	Partial injury but production greatly decreased
			LR	1	1: 4,000	800	15	18.7	0	0	0	0	2	2	2,200§	0	900	600	33.3	Satisfactory cure

*B=Before treatment; A=after treatment; O=test negative; +=test positive.
†200 cc. of this volume given as a preliminary rinse.
‡Teat lumen became occluded by scar tissue.
§LR quarter of cow 37 shedding *S. uberis*.

were necessary, the decrease in milk flow was between 65 and 85 per cent.

The results of this experiment indicate that a 1:4,000 aqueous solution of trypaflavin, as used, is too irritating for safety even when left in the udder for only five minutes. On the other hand, a 1:10,000 concentration is as effective as the stronger solution in removing the streptococci, while it is much less irritating. It may be used with safety and left in the udder for from five to 15 minutes, depending upon the severity of the case.

RESULTS WITH CONCENTRATED SUGAR-TRYPAFLAVIN SOLUTION

In this phase of the investigation, an effort was made, at first, to follow Steck's method III as closely as possible. It was soon found that the use of two separate concentrations of trypaflavin was time-consuming and required additional vessels for preparation of the solutions. It was felt that if the method could be modified to save time and equipment without reducing the efficiency of the treatment in eradicating the infection, it would facilitate its application when a large number of quarters were to be treated. The initial infusion of a dilute aqueous solution of trypaflavin, as used by Steck, was, therefore, dispensed with and only one solution, consisting of a 1:1,500 concentration of trypaflavin in a 20 per cent sucrose solution, was employed. This was used as follows: 200 cc. was infused into the milked-out quarter as a preliminary rinse and, after a short, light massage, this was milked out and replaced by 400 cc. of principal infusion, which was left in for five minutes. Both the preliminary and principal infusions were milked out into a graduated container and the milking was continued until a total of 600 cc. was regained or no more fluid was obtainable. By massaging the quarter toward the teat while milking, the entire infused volume could be regained in 98 per cent of the cases. When a large number of quarters were to be treated, milk bottles marked at the 600-cc. level were used into which to milk the infused fluid.

The infusion fluid for the modified-Steck method was prepared as follows: 0.4 Gm. of trypaflavin was dissolved in 450 cc. of boiled, filtered tap water, after which 150 cc. of a highly concentrated sugar solution was added to bring the total volume to 600 cc., the amount sufficient for treatment of one quarter. The concentrated sugar solution was prepared in advance by dissolving the sugar in hot water in the ratio of 120 Gm. (4 oz.) of household sugar in water sufficient to give a volume of 150 cc. of sugar solution. Several liters of stock solution were prepared at a time and bottled in amounts sufficient for treatment of one, four or more quarters. After bottling, the sugar solution was placed in a steam bath, at a temperature of 100° C., for one-half hour.

The modified-Steck method was used in a herd of Jersey and Jersey-Holstein crossbred cows, which will be referred to as herd H. The breeding program in this herd was such that the majority of cows freshened during the months of October and November. At the time of calving, a number of cows showed symptoms of acute mastitis, after which the disease appeared to spread rapidly to other cows. In several cases the symptoms were so severe that the owner deliberately dried the cows up after only one or two months of lactation. In February a preliminary survey of the herd revealed that 45 out of 77 cows were shedding *S. agalactiae* from one or more quarters. At this time 30 of the infected animals were lactating, while 15 were dry. The herd was visited once a week from March to June, inclusive, and once a month thereafter. All of the lactating and two of the dry cows were treated by the modified-Steck method. Only the infected quarters were infused, of which there were 48 lactating and 7 dry. The 13 remaining dry cows were treated with a 1:10,000 aqueous solution of trypaflavin, which was left in the udder overnight. The data on the latter animals will be presented at another time.

No animals were eliminated from the herd prior to treatment; thus, all infected quarters were treated, regardless of the

TABLE III—Results with a Concentrated Sugar-Trypaflavin Solution in the Eradication of S. Agalactiae by Udder Infusion

Palpation Score Before Treatment	Number of Quarters	Number of Quarters from Which S. agalactiae Was Eradicated by			Number of Quarters Not Cured by 3 Infusions	Number of Quarters Positive to Strip Cup*		Number of Quarters Positive to B C P*		Palpation Score of Quarters After Treatment†		
		One	Two	Three		B	A	B	A	No Change	Improved	Injured
1	4	4	0	0	0	1	0	2	3	3	0	1
2	11	8	3	0	0	1	0	3	2	2	6	3
3	20	15	3	0	2	5	0	12	11	10	6	4
4	20	11	6	1	2	8	0	17	10	13	7	0
Totals	55	38	12	1	4	15	0	34	26	28	19	8

*B=Before treatment; A=after treatment.
†No change=Palpation score remained same after treatment; Improved=quarters softer and more pliable after treatment than before; Injured=quarters became firmer or partially atrophied after treatment.

severity of the infection. The infected animals were segregated from the remainder of the herd until pronounced cured. During the course of treatment the milking machines and milkers' hands were dipped in chlorine between the milking of cows in both the clean and infected groups. The udders were of small to medium size, and it was found in a number of instances that the 400 cc. of principal infusion distended the quarter quite tensely. A few partially atrophied quarters were found that would not hold 400 cc. The classification of quarters by palpation before treatment, the number of treatments required to eliminate the streptococci, and the effect of treatment upon the strip-cup test, the BCP test and palpation score are shown in table III.

It will be observed that four quarters showing no fibrosis responded to one treatment and that one of these was firmer after treatment than before. Of eleven quarters having a palpation score of 2, eight responded to one treatment, while three required two treatments, and after recovery two of the eleven quarters had the same palpation score; six had become softer and were given a lower score, while three had become firmer and were regarded as having been partially injured by treatment. Of 20 quarters showing a distinct fibrosis, 15 were cured by one treatment, three by two treatments, and two were pronounced incurable after three infusions had been made. Ten of these quarters showed no change on palpation after treatment, six were softer and four became firmer. Of 20 quarters exhibiting a marked firmness prior to treatment, eleven were cured by one infusion, six by two, one by three infusions, and two were pronounced incurable after three treatments had been given. Thirteen of these quarters remained firm and seven became softer and were given a lower palpation score after treatment.

Of the total of 55 quarters given treatment, *S. agalactiae* was eradicated from 38, or 69 per cent, by a single infusion; from 12, or 21.8 per cent, by two infusions; and from one quarter, or 1.8 per cent, by three infusions. Four quarters, or 7.2 per cent, were pronounced incurable after three treatments had been given. On an individual cow basis, 30 animals, or 93.7 per cent, were freed of *S. agalactiae* by treatment with the modified-Steck method, while two cows, or 6.3 per cent, were removed from the herd as incurable. Fifteen quarters, or 27.3 per cent, were positive to the strip-cup test prior to treatment and all became negative following treatment. Thirty-four quarters, or 71 per cent, were positive to the BCP test prior to treatment and, of this number, 26, or 54 per cent, continued to produce alkaline milk after treatment for the remainder of the lactation period.

The BCP tests on the two dry cows are not included in the table since the fluid from dry udders is normally alkaline. The effect of the infusions upon the udder

tissues is indicated by comparison of palpation scores before and after treatment. Twenty-eight quarters, or 50.9 per cent, were scored the same on palpation after treatment as before; 19 quarters, or 34.5 per cent, were softer and more pliable after treatment, while eight quarters, or 14.5 per cent, developed either an increase in firmness or some atrophy after treatment and were, therefore, considered as having been injured. In the case of these latter quarters, it was found that they were small or partially atrophied before treatment and the infusion fluid had filled them tensely. It was concluded, therefore, that the volume of principal infusion should be lessened when treating that type of quarter. However, several of these cows have recently freshened again. From all indications, the injury was not permanent, for their udders are symmetrical and all quarters are functioning normally.

In another herd, consisting of Holsteins having medium-size to large udders, in which the modified-Steck method is now being used, it has been found that 400 cc. frequently is not sufficient to fill the cistern. In the majority of the cases, more than one treatment has been required to eliminate the streptococci. With these larger udders, the volume of principal infusion used in the repeat treatment has been increased to 600 and even 800 cc., depending upon the size of the udder, and it has been left in for ten instead of five minutes without producing any visible ill effects. The success of the modified-Steck method in herd H is attributed to the fact that the udders were not large and the 400 cc. of principal infusion actually filled the cistern region.

The benefits derived from treatment in herd H far outweigh any losses which may have resulted from a decrease in milk production during the period of treatment or from partial damage to some of the quarters. By intensive treatment of all infected quarters, *S. agalactiae* was apparently eradicated from the herd within a period of three months. Not a single case of mastitis has appeared during the five-month period which has elapsed since the herd was pronounced free from infection with *S. agalactiae*. Thus, by means of the infusion therapy, a herd having a high incidence of infection with *S. agalactiae* has been rendered free within the space of a few months. To accomplish this end through a program of segregation and elimination of infected cows would require many months.

CONCLUSIONS

1) A 1:4,000 aqueous solution of trypaflavin, as used in these experiments, was found to be too irritating to be employed with safety in the infusion therapy for streptococcic mastitis, even when left in the udder for only five minutes.

2) A 1:10,000 aqueous solution of trypaflavin is as efficient as a 1:4,000 concentration in eradicating the mastitis streptococci from udder tissues, and is much less irritating. With lactating cows, from 800 to 1,200 cc. may be infused per quarter and left in for from five to 15 minutes with safety. In dry udders it may be left in overnight.

3) The udder tissues have a marked capacity for retaining infused aqueous solutions. The efficiency of eliminating the streptococci and the degree of irritation are influenced by the amount of trypaflavin retained by the udder. Since the amount retained by individual quarters varies considerably, the results obtainable with aqueous solutions of trypaflavin are unpredictable.

4) By using a hypertonic (20 per cent) sugar solution as a vehicle for the trypaflavin, it is possible to milk out, after a lapse of from five to ten minutes, a volume of fluid equal to that infused in as high as 98 per cent of the cases. The sugar holds the fluid in the duct and cistern spaces and thus prevents its absorption by the tissues.

5) By employing a hypertonic sugar solution as a vehicle, it is possible to use a much greater concentration of trypaflavin without damaging the udder tissues, and in this manner the efficiency of the treatment is increased.

6) The mastitis streptococci for the

most part localize in the gland cistern and associated large ducts. By treating the cistern region intensively with a 1:1,500 concentration of trypaflavin in 20 per cent sucrose solution, as high as 69 per cent of quarters infected with *Streptococcus agalactiae* were cured by one infusion and as high as 93 per cent by two infusions.

7) Since the capacity of the cistern region of a quarter varies with udder size, it may be necessary to vary the volume of fluid infused from the 400 cc. recommended by Steck in order to obtain maximum efficiency in elimination of the streptococci.

Color Photography and Its Application to Veterinary Medicine*

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THE FINDINGS of archaeologists indicate that visual education had its inception with early civilization as displayed by the inscriptions and designs found in ancient caves and tombs. The hieroglyphics of the Egyptians demonstrate, as does this ancient picture-writing, that these early pictures were used as a means of conveying information, either as a matter of record or of demonstration.

With the comparatively recent development of precision 35 mm. cameras and compact, easily manipulated projectors, visual demonstration of the most desirable type, *viz.*, photography, has become accessible at moderate cost. Photography largely displaces models, charts and blackboard drawings because of its accuracy and present day availability. In many scientific fields, not excluding veterinary medicine, monotone photographs have long served as a medium of instruction, record, analysis and identification.

COLOR

Today color, as in applied art, has inconspicuously entered into every-day living. Aside from the esthetic attributes of color, its usefulness to science is generally accepted but not appreciated for its full value. Color patterns aid in the studies of inheritance, in the identification of specimens, in the interpretation of laboratory procedures, and in the diagnoses of disease. Combine modern color photography with the science of veterinary medicine and at once a new and constant source of demonstration material is available. When color is added to form and motion (of motion pictures) education by visual perception in the form of projected images can be excelled only by viewing of the actual specimens. Add natural colors to the lantern slide pictures of a gross pathologic condition or to a histologic section and at once the inadequacy of monotone pictures is apparent.

Verbal descriptions from even the most fluent of public speakers produce a wide variety of mental impressions on different individuals. Interpretation of size, form, shape and color even when expressed with technical terminology is often confusing. In visual demonstrations the accuracy of the description is not dependent on the personality or mood of the speaker, and the range of mental impressions by the audience is narrowed.

More important, however, is the evident dearth, in the lecture room, of specimens typical of the subject matter under discussion. At the present a case of generalized bovine tuberculosis, for example, is seldom available for demonstration purposes in the lecture room. Many diseases, important to the veterinarian, are never seen until the first field case is encountered. With present day photographic equipment this awkward and undesirable situation is unnecessary. Clinical and autopsy photographs, in natural color, of cases of infec-

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tious and sporadic maladies would greatly stimulate new enthusiasm and interest in the practitioner, laboratory worker, and student, and incidentally in the instructor.

EQUIPMENT AND METHODS

Heretofore, the standard equipment for photography included the plate-back camera with extension bellows for close-up work, and a projector of massive proportions which used an arc light as the source of illumination. The size, bulkiness and inconvenience of this equipment predisposed it to disuse even though slides may have been available for demonstration. While such equipment is excellent for its purpose it is at present not popular as a means of recording and demonstrating images in natural color due to the high cost of the color film in the large plate sizes. In projection work, there is no necessity for a slide larger than 2″x2″, accommodating 1″x1½″ transparencies as taken on the standard 35 mm. film.

Arc light projectors must be avoided in color film projection because they distort and fade the colors, and the colors and film are gradually destroyed by the intense heat. Depending largely upon the wattage of the incandescent lamp used in the projector, the 35 mm. film may be enlarged to theatre-size proportions on the screen should the size of the audience warrant such a procedure. The present day projectors are so compact and small that they may be carried in the coat pocket. Kodachrome film, as processed by Eastman, is returned with transparencies in individual pasteboard mounts, but for permanency the transparencies should be mounted between 2″x2″ (50 mm.) glass slides and bound with tape. If not subjected to excessive heat when projected or while in storage these color transparencies will retain their brilliance for several years. While Dufaycolor film is available in roll form for use in any standard size camera, the smaller, standardized size of 35 mm. film is recommended because of the low cost of materials. The 35 mm. size is standard for projection equipment and a small amount of space is required for storing the slides.

A wide range of equipment is offered in 35 mm. compact (so-called candid) cameras, the price being largely dependent upon the speed of the lens and the adaptability of the camera to accessory equipment. In the lower price range is an American-made camera popular because of the accessories available for close-up as well as photomicrography. The well-known foreign made cameras, with their precision workmanship, wide adaptability and fast lenses, are widely used. One such 35 mm. camera combines the merits of the reflex-type viewing mechanism with delayed-shutter action up to 12 seconds. In any case, a focal-plane type of shutter is desirable because it allows removal of the lens for introducing special equipment. A focal-plane shutter simulates a window shade pulled across the emulsion surface of the film. Extension tubes, when introduced between the focal plane and the lens, make possible photography at distances from 30 down to 2¼ in. In the latter case the subject is natural size on the film. For example in demonstrating the eye lesions in avian lymphomatosis, a picture made at 2½ in., when projected 25 ft., yields a screen image of the eyeball 20 in. in diameter. For close-up work a ground glass focusing device is necessary for a critical focus on the subject. Two types are available, either a sliding or a rotary stage; both are satisfactory. This stage is supported on a vertical upright and may be raised or lowered for obtaining the desired proportions as viewed on the ground glass.

A contrasting background which does not detract from the specimen is desirable, and for medical specimens black velvet cloth is suitable. Dark specimens may be photographed on a lighter background. A grayish blue flannel cloth provides an excellent material for this purpose. A clear plate glass is used to support the specimens with the desired background under the glass. The glass is cleaned following each

exposure, as stained backgrounds are not desirable.

EXPOSURE

In distances as close as 18 in. the photo-electric light meter may be used to determine the correct time and size of diaphragm opening. However, for close work, that is from 18 down to 2½ in., a larger diaphragm opening or a longer exposure is required. In exposing Kodachrome A film, using two No. 2 photoflood lamps in reflectors as the light source, high-lights or reflections are evident when photographing the moist, glistening surfaces of fresh specimens. Diffusing screens of tracing cloth will largely eliminate high-lights, as will also the polarizing filters now available. However, tracing cloth is somewhat bluish itself and is therefore not particularly suitable for color photography, as it may distort the natural color. Beaded glass as used in doors is also satisfactory for diffusing the light rays. In this connection it is considered good practice to use the lens shade (sun shade) to prevent stray light from directly entering the lens of the camera.

In photographing specimens under water for the purpose of avoiding high-lights, the free blood of fresh tissue specimens imparts a reddish tinge to the water and it therefore may not be practical. Also, the refraction of light rays causes the image to appear flat, thus losing perspective. In general it should be stated that the smaller diaphragm opening used the greater will be the depth of focus, and the more pronounced the three-dimensional effect.

In setting up photographic equipment a dustproof room is desirable although not a necessity. Since artificial illumination is used for most consistent results, there should be a minimum of natural daylight entering the room. Kodachrome Type A film is adjusted to photoflood light and daylight causes predominance of blue in this film.

A practical filing and indexing system has been described by Lamoreaux.[1] It furnishes a means of indexing by a cross system as to subject matter. A 3″x5″ index card should be made for each slide giving the picture number, specimen number, subject title, and a brief case history and description of the morbid anatomy. The reverse of the card may carry the data concerning exposure factors as distance, speed, diaphragm opening, light intensity and equipment used. By such a record it is possible to obtain correct exposures at close distances by referring to correctly exposed pictures and tabulating the data.

CONCLUSIONS

Color photography, especially of pathologic changes, creates new enthusiasm when used in the classroom and forms an accurate and accessible means of instruction and demonstration. Color photographs are a distinct advancement over the monotone pictures in that they display color for comparative and diagnostic studies. The slides are relatively inexpensive and easy to file.

[1]Lamoreaux, W. F.: A system of indexing 2″x2″ slides. Sci., lxxxix (1939), pp. 567-568.

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